A.V. Palladin

BIOCHEMISTRY OF THE NERVOUS SYSTEM

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BIOCHEMISTRY OF THE NERVOUS SYSTEM

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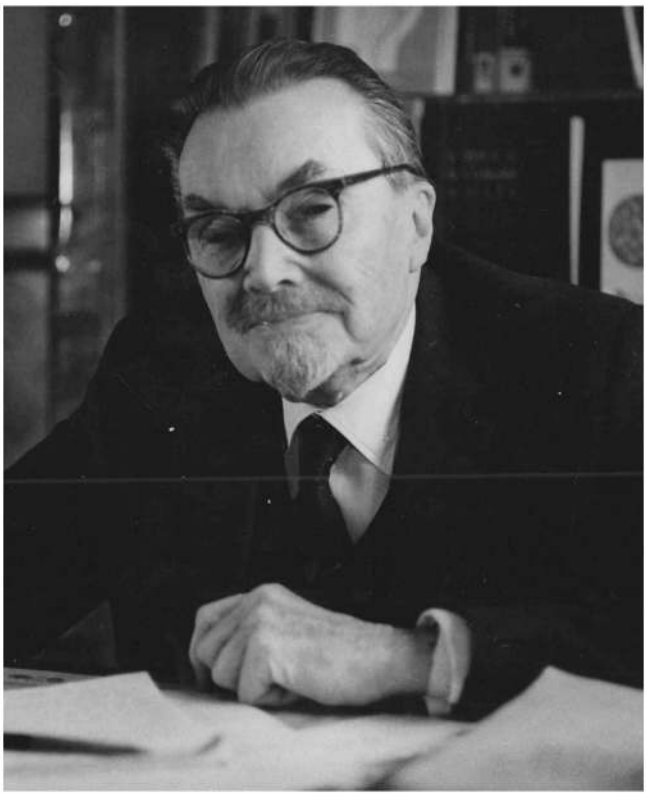
TABLE OF CONTENTS

	Page
Preface	v
The Metabolism of the Brain in Various Functional States	1
Problems in the Biochemistry of the Nervous System	25
Biochemistry of the Brain	37
Biochemical Characterization of Functionally Different Segments of the Nervous System	60
The Use of Radioactive Isotopes for Biochemical Studies of the Nervous System	72
Proteins of the Nervous System, Their Metabolism and Role in Nervous Activity	87
Brain Metabolism during Hibernation	105
Localization of some Enzymes in Subcellular Fractions of the Brain and in Various Protein Fractions Obtained by Electrophoresis	110
Brain Biochemistry and Psychochemistry	119



PREFACE

This edition contains papers devoted to various problems in the biochemistry of the nervous system. The majority of them consists of revised and supplemented scientific lectures which I delivered at various biochemical conferences and symposia held in the Soviet Union and abroad. Some papers, such as the first, third, and sixth, are comprehensive reviews of the researches of foreign and Soviet investigators on the metabolism of the nervous system, and especially of the brain in various functional states. The second paper deals with biochemical problems of the nervous system. One paper characterizes various sections of the nervous system, another describes the use of radioactive isotopes in this area. Next follow papers dealing with the nervous system of hibernating animals, the activity and localization of enzymes of various subcellular cerebral fractions (separable by electrophoresis in agar gels), and the use of neurotropic drugs to elucidate the metabolism of the higher divisions of the brain endowed with psychic activity. Most of the data presented were obtained by the author and his collaborators in the Laboratory of the Biochemistry of the Nervous System of the Institute of Biochemistry at the Academy of Sciences of the Ukrainian SSR.

Academician A. V. Palladin

*THE METABOLISM OF THE BRAIN IN VARIOUS FUNCTIONAL STATES**

Pavlov's school of physiology is based on the principle of unity and plasticity of the living organism, in its interactions with the changing environment, which affects its state of development as well as the changes of functional properties and form in both ontogeny and phylogeny.

According to I. P. Pavlov, . . "the living organism is an extremely complex system consisting of numerous interlinking parts, comprising an integrated entity which exists in equilibrium with its environment" /1/.

In higher organism, and especially in man, the integrity of the organism and its interactions with the external and internal millieu are controlled by the central nervous system and by its youngest phylogenetic part — the cerebral cortex. According to Pavlov the nervous system integrates the activity of the organism and controls its interaction with the environment with which it is in equilibrium. Pavlov's concept agrees with the teaching of Engels, who states that the main feature of vertebrates is the central position occupied by their nervous system, which organizes and directs bodily functions according to the arising need /2/.

Any living process, be it tissue metabolism or the most complex psychic activity, is regulated by the cerebral cortex.

Metabolic studies of the brain are indispensable for the understanding of its nervous activity. Pavlov emphasized that a true theory of all nervous phenomena can be obtained only upon studying the physicochemical processes taking place in nerve tissue /3/. He further stressed that understanding the processes of excitation and inhibition, which are characteristic of all nervous activity, depends mainly on our knowledge of physics and chemistry. Thus, the importance of metabolic studies of the nervous system and especially that of the brain, becomes apparent. The objective is to study the role of metabolic processes and their effect on the various functions of the nervous system.

The ultimate goal, as yet unattained, is to define biochemically the various functions of the nervous system. Pavlov's "functional biochemistry" entails the investigation of metabolic processes of the brain and their effect on nervous activity.

Engels proposed the modern materialistic concept of metabolism as the basis of all living processes. He wrote: "Life consists of the turnover of proteins. With the cessation of protein turnover life ceases" /4/.

We (Palladin et al.) of the Institute of Biochemistry of the Academy of Sciences of the Ukrainian SSR have been engaged for several years in biochemical investigations of the nervous system, with particular emphasis on the brain in its various functional states.

* Revised article from Vestnik Akademii Nauk SSSR No. 10:37-62 1952.

In our studies we have attempted to determine the chemical composition of various functionally different brain sections and to ascertain whether the development of a function may affect the chemical composition of a given section. We also wished to determine whether there exists a correlation between the function of the various brain sections and their chemical composition. We have attempted to show how various factors which affect the activity of the brain as a whole may influence metabolic changes in various brain sections. Finally, we have attempted to determine whether metabolic processes in the brain, such as carbohydrate metabolism, are similar to those in other tissue /5/.

We first focused our attention on the protein metabolism of the brain as a function of its activity. The importance of proteins to life has been clearly shown by Engels, who wrote: "Wherever we encounter life, we find it connected with some protein; wherever we find any protein which is not in the process of decomposition, we encounter life" /6/.

There is no doubt that proteins play an important role in the central nervous system. A. Ya. Danilevskii was one of the first to recognize the importance of proteins in the function of the brain. In his paper, "Phosphoproteins of the Brain" (Fosforistye belki mozga) published in 1891, he wrote: "Modern biological chemistry has firmly established the fact that proteins play a central role in the life processes of the cell. Life in all its manifestations depends mainly on the presence of proteins with their characteristic properties. Although other organic compounds may also play a role in the activity of the cell, its biological function is due almost exclusively to the presence of a variety of proteins" /7/.

D. Petrovskii, B. Slovtsov, and A. Lents have shown that different regions of the nervous system differ in their protein and lipid contents. The more complex the function of the region, the higher its protein content. According to Petrovskii (1873) cerebral gray matter contains 55.3% protein, whereas white matter contains only 24.7%. Lents (1919) found 42.2% protein in the gray matter of the brain, and only 39% in the gray matter of the subcortical ganglia. Slovtsov and Georgievskaya (1922) reported the following protein concentrations in various regions of the nervous system: the spinal cord — 31%, the sciatic nerve — 29%, the white matter of the cerebral hemispheres — 33%, and the cerebral cortex — 51%. Thus it is seen that the gray matter of the cerebral cortex is richest in protein. This is followed by the white matter, then by the spinal cord, and finally by the peripheral nerves. These data establish the important role of proteins in the central nervous system.

There is a direct correlation between protein and water content in the central nervous system. However, lipid content varies inversely with protein concentration. Thus, the peripheral nerves have the highest lipid content followed by that of the spinal cord. The lowest lipid content is found in the brain. The white matter of the brain, which consists of nerve fibers, has a higher lipid content than the gray matter (the cortex), which consists mainly of nerve cells.

We studied the protein, water and creatine contents (the latter is a nitrogenous compound of great importance in muscles) in brain sections of cows, dogs, rabbits, rats, guinea pigs, pigeons, and lizards, and found /8/ that the distribution of these substances is identical in all these phylogenetically different animals. The highest protein and water contents were found

in the gray matter of the cerebral hemispheres, followed by the cerebellum and the white matter. The creatine content was highest in the cerebellum, followed by the cortex, the white matter of the cerebral hemispheres and, finally, by the corpus callosum.

These differences in protein, water, and creatine contents are most pronounced in mammals, which have the most highly developed central nervous system. They are less pronounced in birds, whose nervous system is less differentiated.

The highest creatine content was found in the brain of frogs, followed by that of lizards, birds, rats, guinea pigs, dogs, cats, and lastly, cows. Thus, there is a gradual decrease in creatine content from the frog to the cow (the fish are an exception).

In order to confirm the conclusion that there is a correlation between nerve function and protein content, we studied the chemical composition of various sections of the central nervous system which were similar or closely related histologically but differed functionally and phylogenetically. These sections included the gray matter of the cerebral cortex, the subcortical ganglia, the cerebellum, and the spinal cord. The gray matter of these sections consists of nerve cells, which means that they were closely related histologically. However, they differ in function and phylogeny.

Studies on the structure of these sections of the gray matter have shown that they differ in chemical composition /9/. They gray matter of the cerebral hemispheres, which is philogenetically the youngest region (i.e., belonging to the later stages of evolution), and functionally the most complex, has the highest protein content. The gray matter of the cortex of the cerebellum and that of the subcortical ganglia contains less protein, while that of the spinal cord has the lowest protein content.

The distribution of lipids is entirely different. The gray matter of the spinal cord, which is phylogenetically the oldest and functionally the least complex region, has the highest content of phosphatides and cholesterol, while the gray matter of cerebral cortex has the lowest content.

These results show that proteins play an important role in the central nervous system since the most complex and phylogenetically the youngest regions of the nervous system are the richest in proteins. They also show that lipids are not characteristic for highly differentiated structures and apparently do not play any specific role in the most complex regions of the brain. Earlier, however, when the brain or the spinal cord were studied as an entity, and it was shown that brain tissue was richer in lipids than any other tissue, it was concluded that lipids were the characteristic compounds of nerve tissue and that they were specifically linked with functions of the higher brain regions.

The chemical composition of the autonomous nervous system differs from that of the central nervous system. Likewise, the peripheral ganglia of the sympathetic and parasympathetic nervous systems, which differ in function, also differ in chemical composition.

The chemical composition of the spinal cord ganglia, of parts of the autonomous nervous system (the peripheral ganglia and sympathetic trunk) and of the efferent pathways of the peripheral nervous system were studied. These studies showed that here also the phylogenetically youngest regions are the richest in nitrogenous matter and in water /10/. The peripheral nerves, which are phylogenetically the oldest, have the lowest protein

content, while the roots of the spinal cord, phylogenetically younger, are richer in protein and cholesterol. The youngest region, the sympathetic trunk, consisting mainly of nonmyelated fibers, has the highest protein content. The oldest region, the ganglia of the spinal cord, is the richest in cholesterol, lecithin and cephalin. In other respects it is similar to the ganglia of the autonomous nervous system.

These studies also showed that the peripheral nerves, which are anatomically a continuation of the anterior and posterior roots of the spinal cord, differ from the latter in chemical composition; also, that the axial cylinders of the nerves, which comprise the processes of nerve cells, differ in chemical composition from the nerve cells proper. In all cases a difference in function correlated with a difference in chemical composition.

The chemical composition of the entire brain and of its individual parts depends not only on the phylogenetic development, as shown by the results of studies on the chemical composition of the brain of animals of different phylogenetic development, but also on the stage of embryonic development. Our studies showed /11/ that during embryonic development, the protein, creatine, and water contents of the brain and of its various parts gradually decrease. In some animals, such as cows, the content of these three substances reaches the level characteristic of the adult brain during the seventh month of embryonic development. In others, such as rabbits and guinea pigs, the composition of the brain continues to change during the first month after birth, attaining the characteristic adult level only at the end of the first month.

Studies of the brain of cow embryos have shown that its various regions differ in chemical composition starting with the third month of embryonic development. We found that at that time the cerebellum had the highest creatine content and the brain stem had the lowest protein and water contents as compared with other parts of the nervous system. We did not study the brain of embryos at earlier stages of development. These results have discredited the prevailing view that there are no differences in the chemical composition of brain components prior to myelination (that is, until the final stages of embryonic development) or even till birth. We found that embryonic brain divisions differ in their chemical composition long before myelination.

Our studies on the effects of vitamin deficiency also showed a correlation between functional changes and the biochemical processes taking place in the brain. When pigeons were deprived of vitamin B_1 they developed polyneuritis, with characteristic functional aberrations of the nervous system. It was found that the creatine content of the brain changed proportionally with the degree of aberration — the more severe the aberration the higher the content of creatine. The change in creatine metabolism was most pronounced during the spastic form of polyneuritis /12/.

In experimental scurvy, another vitamin deficiency disease, functional changes of the nervous system differed from those observed in polyneuritis. Therefore, no change was observed in the creatine content or metabolism in the brain /13/. Thus, creatine metabolism in the brain is disturbed only in those vitamin deficiencies accompanied by a disturbance in brain function, when the central nervous system is in a state of excitation.

During starvation the protein metabolism of the brain is altered. Degradation processes decrease in the gray matter of the cerebral

hemispheres and increase in the white matter /14/. Brain tissue becomes richer in creatine and the water content increases, causing the weight of the brain to remain unchanged. This led earlier investigators to the erroneous conclusion that brain metabolism did not change during starvation. Aside from alterations in nitrogen and protein metabolism, starvation also produces disturbances in carbohydrate and lipid metabolism.

Brain creatine metabolism is dependent on the seasons of the year. Experiments with pigeons showed that in the spring the creatine content of the brain differs from that in the fall. We also found that the brain of pigeons killed in March or the beginning of April contained more water and had a higher rate of proteolysis than of those killed in June.

Similar data were obtained in experiments with rabbits. The brain of rabbits contained more creatine in the fall than in spring. The same was found to be true of rabbit embryos. Their water content also differed /15/.

In all of these experiments the total protein content in the different brain components was determined. It may be assumed that functionally different regions of the central nervous system contain different amounts of protein which are specific for the given region. Therefore, we attempted to fractionate the brain proteins and study the individual fractions.

In 1873 D. Petrovskii isolated several protein fractions (albumin and globulin) from the brain. Danilevskii also paid much attention to the study of brain proteins. He was the first to develop a method for the fractionation of brain proteins. In 1919 he isolated neuroglobulin from brain tissue and showed that it contained phosphorus. He also isolated neurostromin (1919). The method developed by Danilevskii for the fractionation of brain proteins was used by him and other Russian scientists, such as N. Shkarin, and A. Lents, to determine the content of neuroglobulin and neurostromin in functionally different sections of the human brain and of the brains of various animals of different ages.

In spite of these studies, till now the area of brain tissue proteins has not been studied sufficiently. This is probably due to the difficulties encountered in studies of this kind. Proteins of nerve tissue are bound to lipids, forming lipoprotein complexes. In addition, these proteins form extremely unstable colloids.

In order to study the protein composition of the various brain sections, we first decided to separate the proteins extracted from brain tissue into a small number of fractions, using methods in which protein denaturation was minimum and separation from lipids was complete.

The proteins were extracted from brain tissue with water, a 4.5% solution of potassium chloride, pH 9.1, and a 0.1 N solution of sodium hydroxide. In this manner we obtained three protein fractions, which differed from each other in their isoeletric points /16/. When experimental conditions were kept constant we found that we obtained similar amounts of protein fractions in successive experiments. Thus, the quantitative determination of protein content became feasible.

We found that the protein content of the gray matter differed from the white, indicating that the white and gray matter of the brain have different protein compositions.

Table 1 shows that in the white matter water extractable fractions comprise a little more than 19% of all nitrogenous matter while in the gray matter they comprise about 31%. Thus the gray matter is richer in

water-extractable proteins, such as albumins and probably globulins. The white and gray matter are similar in their content of proteins extractable with potassium chloride and sodium hydroxide, but differ sharply in their content of proteins which remain as insoluble residue.

TABLE 1. The protein content of separate fractions in the white and gray matters of the brain

Protein fraction	Protein nitrogen, % of total nitrogen	
	White matter	Gray matter
Extracted with water (albumin and globulin mixture)	19.6	31.0
Extracted with 4.5% KCl (pH 9.1)	23.6	28.3
Extracted with 0.1 N NaOH	34.7	36.3
Insoluble residue	22.0	5.0

Thus, the gray and white matters of the brain differ from each other not only in total protein content but also in the nature of their proteins. The gray matter is richer in water-soluble proteins and poorer in insoluble residue.

When it was found that muscle myosin displayed adenosine triphosphatase activity /17/, investigations were begun to see whether this activity coul be found in the structural proteins of other tissues. Such proteins have also been isolated from brain tissue /18/, and their hydrolytic activity on adenosine triphosphate (ATP) has been elucidated.

Since the structural proteins of a number of tissues (liver, kidney, etc.) are mixtures of nuclear and cytoplasmic nucleoproteins, their adenosin triphosphatase activity cannot serve as a criterion of their purity. Therefore, we found it necessary to study in detail the structural proteins of the brain with respect to their chemical composition and enzymatic activity. We found that structural brain proteins are an artifact obtained during protein extraction — that they consist of a mixture of nuclear deoxyribonucleoproteins and cytoplasmic and nuclear ribonucleoproteins — which contain adsorbed enzymes and lipoproteins. Adenosine triphosphatase activity did not characterize brain proteins any more than did the activity of other enzymes such as amylase, phosphatase and succinic acid dehydrogenase.

It is clear that neither the amount of this protein nor its enzymatic activity can serve as a criterion for studying the functional states of the nervous system /19/. For such studies the proteins should not be isolated from the whole brain tissue but from its isolated morphological elements. Likewise a detailed study of brain tissue proteins and in particular of ribonucleoproteins, is of great importance since such proteins play a significant role in the activity of the nervous system.

Numerous studies have confirmed the significant role of ribonucleic acid and ribonucleoproteins in living organisms and in the nervous system. It has been shown that the ribonucleic acid content of nerve cells decreases sharply following vigorous cell activity or after excision of the corresponding axons. It also changes during nerve regeneration

(Khiden, Bodian). In these investigations the microscopic method was used. Biochemical studies have not yet been conducted on ribonucleoproteins of the nervous system. There is no doubt that such studies are of great importance.

We isolated a ribonucleoprotein from the gray matter of brain and studied some of its properties /20/. This brought us a step closer to our objective of finding a correlation between the physiological activity of the nervous system and ribonucleoprotein metabolism. It became apparent that the cytoplasmic ribonucleoprotein is a genuine nucleoprotein with a strong bond between the ribonucleic acid and the protein (the ratio of N to P is 8). The protein fraction of this nucleoprotein is a lipoprotein containing numerous amino acids, among them arginine, tryptophan and tyrosine.

We determined the ribonucleoprotein content in the cerebral hemispheres and cortex of rats, rabbits, and cows. Some of the results of these studies are presented in Table 2.

TABLE 2. Content of the soluble cytoplasmic ribonucleoprotein in the brain of rats and rabbits in percent of fresh tissue

Object for examination	Soluble ribonucleoprotein		Ribonucleoprotein phosphorus (% of total phosphorus)	Ribonucleoprotein nitrogen (% of total nitrogen)
	Phosphorus, %	Nitrogen, %		
Cerebral hemispheres of rats				
Average	0.0074	0.057	18.0	3.7
Limits of fluctuation	0.0072-0.0076	0.055-0.060	17.2-18.8	3.6-3.8
Cerebral cortex of rats				
Average	0.0016	0.136	36.7	8.4
Limits of fluctuation	0.015-0.017	0.123-0.152	35.5-39.0	7.0-9.8
Cerebral cortex of rabbits				
Average	0.013	0.10	29.0	6.6
Limits of fluctuation	0.010-0.018	0.084-0.146	24.2-34.3	4.9-9.1

Aside from the cytoplasmic ribonucleoprotein which is soluble in physiological solutions, brain tissue also contains insoluble nucleoproteins, the nucleoproteins of the nucleus. To study the nuclear nucleoproteins it is first necessary to isolate the brain nuclei and to extract from them the nucleoproteins. By using a method /21/ devised for the separation of cytoplasm from nuclei in solutions of appropriate specific gravity we succeeded in isolating the nuclei from the gray and white matter of the cerebral hemisphere of cows and from the whole brain of dogs. We obtained 200 mg of nuclei from 100 g of gray matter. The yield from white matter was lower.

Studies on nuclei from the white and gray matter of cow brains have shown that nucleic acids comprise 21-44% of all organic compounds. These nucleic acids consist of 20-30% ribonucleic acid and 70-80% deoxyribonucleic acid. The nuclei of dog brain contain only about 10-15%

ribonucleic acid. The nuclei of other tissue cells contain considerably less ribonucleic acid than do the nuclei from the gray and white matter of the brain (especially in cows — see Table 3).

TABLE 3. The chemical composition of brain nuclei after lipid extraction (in weight % calculated for nucleic acids and proteins containing about 16% nitrogen)

No. of experiment	Animal	Source of nuclei	Total phosphorus	N:P	DNA %	RNA %	DNA/RNA	Phosphoprotein phosphorus, %	Protein %
2	Cow	From the gray matter of the hemispheres . .	3.25	5.0	21.5	11.0	1.9	0.091	67.5
5		The same	3.60	4.1	26.0	13.4	1.9	0.088	60.6
6		" "	3.50	4.7	19.1	16.3	1.2	0.157	64.6
7		" "	4.03	4.1	27.5	11.4	2.4	0.234	61.6
11		From the white matter of the hemispheres . .	3.00	5.5	27.6	11.8	2.4	0.118	60.5
19	Dog	From the whole brain	3.51	4.8	25.8	3.0	6.6	0.670	70.3

Nuclear nucleoproteins contain ribonucleic acid in addition to deoxyribonucleic acid which exists mainly as deoxyribonucleoprotein. Nuclear nucleoproteins comprise 27-41 of the total weight of the nuclei. The ratio of N to P in the nucleoprotein is 2:2 to 3:5, which differs from that of cytoplasmic ribonucleoproteins (see Table 4). These studies show that the neuroglobulin of Danilevskii is a nuclear deoxyribonucleoprotein and that the neurostromin is a ribonucleoprotein.

Our work on the isolation and characterization of the cytoplasmic and nuclear nucleoproteins of brain presents possibilities for further research in this area. Thus it may be interesting to study the correlation between brain function and the metabolism of individual protein fractions, and particularly of the nucleoproteins.

TABLE 4. The chemical composition of deoxyribonucleoproteins isolated from the nuclei of the gray matter of the cerebral hemispheres of cows (in weight %, calculated as in Table 3)

No. of experiment	Deoxyribonucleoprotein, %	Total phosphorus	N:P	DNA, %	RNA, %	DNA/RNA	Phosphoprotein phosphorus, %	Protein, %
7	41	6.5	2.5	55.6	8.4	6.6	0.124	36.0
8	46	7.6	2.2	62.5	8.1	7.7	0.202	29.4
12	40	4.6	3.5	41.5	3.8	11.3	0.104	54.6

We have already mentioned that the brain regions which are functionally the most complex are the richest in creatine, that the creatine content in the brains of various animals depends on their phylogenetic development, and that in birds the differences in the creatine content of various brain component are very small.

The distribution of creatine in various regions of the brain of rabbits, pigeons, fish and amphibia corresponds to the distribution of the amino acid arginine. Arginine is known as one of the precursors of creatine in the living organism. The arginine content of the hemispheres of the cerebellum, of the cerebral hemisphere, and of the brain stem varies directly with the creatine content. The total arginine content of the brains of various animals diminishes in the phylogenetic series /22/.

Carbohydrates also play an important role in the central nervous system. They are the main source of energy in nerve tissue. Although brain tissue has no considerable reserves of carbohydrates (glycogen or glucose), they nevertheless serve the brain as an important source of energy. The respiratory quotient of the brain equals 1; hence, oxidative processes proceed mainly at the expense of carbohydrate oxidation.

In studying carbohydrate metabolism and its variations with changing brain functions, we attempted to clarify whether carbohydrate metabolism in the brain (glycolysis) involves the participation of phosphorus, as in muscle glycolysis, or proceeds by another path without utilization of phosphorus.

Our studies showed that glycolyis in brain tissue involved, numerous intermediate compounds containing phosphorus. These phosphorylated intermediates were similar to those found in muscle glycolysis. For example, during embryonic development chicken brain is rich in a number of phosphorylated compounds, among them ATP and creatine phosphate, which play an important role in glycolysis. With the further development of the animal the content of these compounds gradually decreases.

Analogous data were obtained by us with rabbit brains /23/. We found that this tissue contains the same phosphorylated compounds as were found in muscle. The composition and content of these compounds change in the course of ontogenetic development. During the first days after birth, rabbit brain contains large amount of adenosine triphosphate (and also creatine phosphate), which decreases and becomes undetectable in the brain of adults. This led former investigators to conclude that brain tissue lacks ATP and because of this differs from muscle tissue. The high percentage of phosphorylated compounds found in the brain immediately after birth is undoubtedly due not only to the morphological features, which change during the active growth of the animal, but also to metabolic changes which occur before and immediately after birth. The intensive glycolytic activity in embryonic brain tissue may also be a factor.

Indeed, we found that the glycolytic activity of brain tissue changed during the different stages of ontogenetic development. Maximum glycolytic activity was found in the brain of embryos, and was somewhat diminished in newborn animals. Glycolytic activity decreases with the growth of the animal, soon reaching the value characteristic of the adult animal brain /24/.

Thus, the level of phosphorylated compounds participating in brain tissue glycolysis is highest when glycolytic activity is most intense. These data confirm that phosphorus participates in the carbohydrate metabolism

of brain tissue and show that glycolysis depends on the presence of phosphorus.

Glycolytic activity is not uniform in all the brain tissue. It is higher in the gray matter (of the cerebral cortex) than in the white matter. Brain tissue utilizes glucose rather than glycogen /25/. In this respect it differs from muscle tissue where glycogen is the source of energy.

We also found /26/ that oxidation-reduction reactions proceed at a higher rate in the gray matter than in the white.

In order to study the carbohydrate metabolism of the brain in greater detail we first undertook to study the enzymes involved in the various glycolytic steps. If we were successful in identifying these enzymes and in establishing their chemical properties and mechanism of operation we would have a clearer understanding of glycolysis in brain tissue. Moreover, we would be able to control the glycolytic process and direct it in the desired paths.

The first glycolytic step, the hydrolysis of the polysaccharide glycogen, is catalyzed by the enzyme phosphorylase. Glucose-1-phosphate is obtained.

Our studies on brain phosphorylase have shown that its phosphorolytic activity is very weak and that its main function is concerned with the synthesis of glycogen from glucose-1-phosphate. We found that phosphorylase catalyzes the synthesis of starchlike polysaccharides (amylase) /27/ which are converted into the branched carbohydrates of the glycogen type by the action of an isomerase, which may be named starch-glycogen isomerase.

Phosphorylase and isomerase from brain tissue can be separated by ammonium sulfate fractionation. Isomerase activity is inhibited by sodium fluoride.

Phosphorylase was discovered in 1936 and was shown to be the catalyst in the hydrolysis of glycogen in muscle and liver, rather than amylase, as had been formerly thought. As a result the presence of amylase in animal tissue was completely rejected. The old data of such Russian scientists as Ossovskii (1919), Slovtsov (1921) and Petrunkin (1922) on the presence of amylase in brain tissue were forgotten. It was assumed that in brain as in other tissues, glucose is not formed from glycogen by the action of amylase, but by the action of phosphatase on glucose-1-phosphate, which is formed from glycogen by the action of phosphorylase.

We showed /28/ that brain tissue contains a very active amylase which catalyzes the hydrolysis of glycogen to form dextrins, maltose and free glucose. Amylase is adsorbed by brain tissue proteins and can be freed from them during autolysis. In this respect it differs from blood amylase which during autolysis loses its activity. Studies on the chemical nature of amylase showed that it is an albuminlike protein, called by us neuroalbumin /29/.

We have shown /30/ that the hydrolysis of glycogen in brain tissue is catalyzed by amylase and that in this respect brain tissue differs from muscle. The synthesis of polysaccharides in the brain is catalyzed by phosphorylase and isomerase.

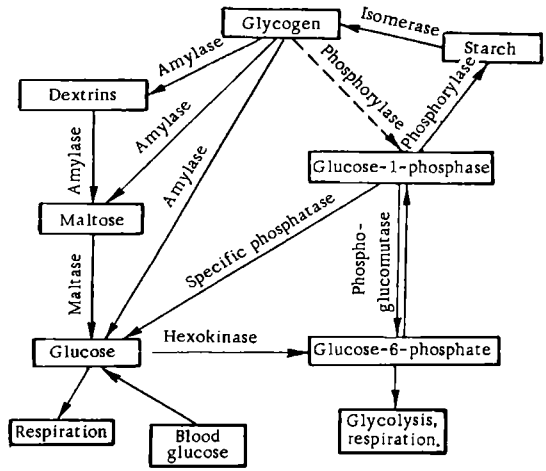
Glucose may be supplied to the brain tissue by the blood stream but can also be formed from polysaccharides in the brain in the presence of amylase. It can also be formed from glucose-1-phosphate by the action of a specific phosphatase present in the brain but not connected with the structural elements of brain tissue. This phosphatase hydrolyzes glucose-1-phosphate to free glucose and phosphoric acid. Brain tissue also contains nonspecific phosphatases which hydrolyze hexose phosphates and sodium glycerophosphate.

How is glucose utilized by the brain tissue? In various tissues the first stage is usually the phosphorylation of glucose, catalyzed by hexokinase.

Geiger, Hussack and Ochoa thought that brain tissue contains hexokinase. However their evidence for its presence was indirect. Hussack thought that hexokinase is present only in the gray matter.

We found /31/ that hexokinase is indeed present in brain tissue where it catalyzes the ATP-dependent phosphorylation of glucose, with the formation of hexose-6-phosphate. Hexokinase could be detected in animals from the first day of life both in the gray and white matter. Its activity was much higher in young animals than in adults. It is possible that this is due to the higher glycolytic activity in the brain of young animals.

The transformation of glucose-1-phosphate formed by phosphorolysis is catalyzed in the various tissues by the enzyme phosphoglucomutase and leads to the formation of glucose-6-phosphate.



Phosphoglucomutase is also present in brain tissue and, as our studies have shown, is present in animals of all ages from birth on. This finding contradicts the data of Shapiro and Wertheimer who reported that this enzyme appears in brain tissue commencing with the 10th day of life.

In the presence of phosphoglucomutase the reaction may proceed in two directions. Glucose-1-phosphate may be converted into glucose-6-phosphate or glucose-6-phosphate may be converted into glucose-1-phosphate. Since brain phosphorylase catalyzes the synthesis of polysaccharides from glucose-1-phosphate, it may be assumed that the pathway from glucose-6-phosphate to glucose-1-phosphate is the main reaction catalyzed by brain phosphoglucomutase.

All of these data lead to the conclusion that in the brain glycogen is hydrolyzed in the presence of amylase to form dextrins, maltose, and glucose. Maltose is hydrolyzed in the presence of maltase, present in brain tissue. Glucose formed from glycogen and glucose obtained from the blood stream are converted into glucose-6-phosphate in the presence of

hexokinase. Glucose-6-phosphate, in the presence of phosphoglucomutase, is converted into glucose-1-phosphate which may then serve as a substrate for the synthesis of polysaccharides. Phosphorylase catalyzes the synthesis of unbranched starchlike polysaccharides which in the presence of isomerase are converted into glycogen.

In the schematic representation of the initial stages of hydrolysis and synthesis of brain carbohydrates /32/ it can be seen that glucose occupies the central position in these reactions. Glucose may serve as a substrate for the synthesis of polysaccharides or as a source of energy through oxidation or glycolysis.

Glucose-1-phosphate is the most important intermediate in the initial stages of carbohydrate metabolism in the brain. It serves as a substrate for the synthesis of polysaccharides as well as for further conversions leading to the formation of glucose or lactic acid (glycolysis or respiration).

Biochemists have recently paid much attention to adenosine triphosphate which is found in almost all animal tissues. It is an energy rich compound which is utilized for synthetic processes and other specific purposes. Hydrolysis of adenosine triphosphate is catalyzed by adenosine triphosphatase with the release of energy which is stored in pyrophosphate bonds. Adenosine triphosphatase has been found in every animal tissue that has been studied. It is also present in brain tissue but has not been closely investigated. We therefore attempted a detailed study of adenosine triphosphatase.

We found /33/ that brain adenosine triphosphatase differs in some of its properties from adenosine triphosphatase of other tissues. In contrast to adenosine triphosphatase of muscle tissue, which is activated by calcium ions, the brain enzyme is activated by magnesium ions. Copper ions at high concentrations inhibit adenosine triphosphatase activity.

We detected in the brain a specific adenosine triphosphatase inhibiting compounds which could not be found in muscle tissue. Since adenosine triphosphatase plays an important role in glycolysis, this inhibitory substance may be of great significance in carbohydrate metabolism.

The subject of inhibitors which participate in the regulation of glycolysis is of great interest. Pavlov had already pointed out that various "anti-enzymes" play an important role in the physiological processes which take place in organs and tissues.

Adenosine triphosphatase activity is highest in the brain of adult animals and lowest in the newborn. With the growth of the animal adenosine triphosphatase activity gradually increases.

Studies on adenosine triphosphatase activity in various brain regions show that it is highest in the cerebellum and gray matter of the cortex, lower in the medulla oblongata, and lowest in the white matter. Thus, the brain regions with the most complex and important functions show the highest adenosine triphosphatase activity (see Table 5).

Aldolase is another of the enzymes which we included in our studies on the carbohydrate metabolism of the brain. This enzyme catalyzes the cleavage of fructose-1.6-diphosphate into two phosphate molecules. All that was known about adolase till recently was that its activity is lower in brain tissue than in muscle (Meyerhoff). Nevertheless aldolase plays an important role in carbohydrate metabolism.

TABLE 5. Adenosine triphosphatase activity in various divisions of the brain (per mg of protein)

Substrate	Brain of cow	Gray matter of the cerebral hemispheres	White matter of the cerebral hemispheres	Cerebellum	Medulla oblongata
Adenose triphosphate	Extract	30	19	30	25
The same	Homogenate	37	16	34	27

After obtaining aldolase in its purified form and studying its properties /34/, we found that its activity is identical in the brains of several animals (rats, rabbits, dogs and cows). We also found that functionally different regions of the central nervous system have different aldolase activities. The highest activity was found in the cerebellum and in the gray matter (cortex) of the hemispheres. It was lower in the white matter of the hemispheres and in the medulla oblongata. Here, too, the brain regions which are functionally the most complex exhibit the highest aldolase activity.

TABLE 6. Aldolase activity in various divisions of the brain (in μg of phosphotriose phosphorus per ml of homogenate, diluted 1:100, per hr at 37°C)

Object	Cerebral hemispheres	Gray matter of the cerebral hemispheres	White matter of the cerebral hemispheres	Cerebellum	Medulla oblongata
Cow	167	183	67	207	90
Dog	187	207	127	233	—

Aldolase activity differs in animals of different ages. In adult rabbits the activity is higher than in embryos or in those one to ten days old (Table 7). This result is somewhat unexpected. Since the highest glycolytic activity is found in animals at early stages of development, it was thought that aldolase activity would be higher in embryos and in newborn animals than that in adults.

Adenosine triphosphatase is also less active in the brain of animals in early stages of development. Is this not due to the higher content of hexose phosphates and adenosine triphosphate in the brain of young animals, as shown in our studies?

The enzymes adenosine triphosphatase and aldolase that have been described in carbohydrate metabolism have different activities in the various brain regions. Functionally complex regions are characterized by a high enzymatic activity (or a high enzyme content). Likewise, according to our data, hexokinase is most active in the gray matter of the cerebral hemispheres, followed by the cerebellum, the medulla oblongata, the white

matter of the cerebral hemispheres, and the spinal cord. An analogous picture was obtained in studies of other enzymes, such as phosphorylase.

Although there are many similarities in the carbohydrate metabolism of brain and muscle tissues, such as the presence of similar phosphorylated intermediates, the presence of analogous enzyme, and similar glycolytic paths which utilize phosphorus, carbohydrate metabolism of the brain nevertheless differs in some respects from that of muscle. This is undoubtedly due to the specific physiological function of the brain. Thus, the hydrolysis of glycogen in the brain is catalyzed mainly by amylase, and phosphorylase activity is directed mainly towards the synthesis of the polysaccharide from glucose-1-phosphate. In these respects brain tissue differs from that of the muscle and other tissues. In the nerve tissue glycogen is synthesized by two enzymes —phosphorylase and isomerase. The main source of energy in the brain is glucose and not glycogen, as in muscle. Brain tissue contains an adenosine triphosphatase inhibiting substance which has not been found in muscle. In other words, the brain contains factors participating in the regulation of carbohydrate metabolism which are absent from other tissues. These specific peculiarities of brain metabolism, connected with the functional specificity of nerve tissue, are not accounted for by foreign authors (such as Meyerhoff) who, proceeding from erroneous methodology, obtain wrong results.

Studies on the properties, the mode of action, and the role of the various enzymes in nerve tissue, depending on the function of the latter, as well as on the enzymatic activity in the functionally different sections of the brain during embryonic and postembryonic development directed our attention to the possibility of altering the activity of various enzymes by altering the environment. The possibility of altering enzymatic activity in the living organism by changing the environmental conditions has been established by Pavlov in his studies on digestion. We have shown that the enzymatic activity of muscles can be altered through training and increased muscular activity /35/. The study of the alteration of enzymatic activity in living organisms under various environmental conditions is one of the most important problems of biochemistry.

TABLE 7. Aldolase activity in the brain of rabbit embryos and of rabbits of various ages

Object	μg of phosphotriose phosphorus per ml of homogenate diluted 1:100	In μg of phosphotriose phosphorus per mg protein
27-28-day-old embryos	66.0	109
One-day-old rabbit	83.5	111
8-day-old rabbit	83.0	112
10-day-old rabbit	107.0	137
Adult rabbits	193.0	187
" "	213.0	187
" "	200.0	206
" "	219.0	206

The feasibility of altering enzymatic activity in the brain by varying the environmental conditions has been shown in the following way /36/. Rabbits and rats, when kept on a sucrose diet for a prolonged time, developed. chronic hyperglycemia; 45-80 days after the commencement of the sucrose diet, we determined the amylase content of the brain and showed that a amylase activity decreased. Analogous results were obtained with animals having alloxan diabetes. Their blood amylase activity increased while that of the brain decreased.

Thus, alimentary hyperglycemia and experimental diabetes can alter the activity of brain amylase. In these instances, due to the changed internal environment, (the high content of blood sugar) polysaccharide hydrolysis plays a lesser role and the activity of the enzymes catalyzing this reaction decreases.

While studying the enzyme catalyzing the hydrolysis and synthesis of glycogen we determined content in the various brain sections and found that it could be detected only in the cortex and cerebellum of healthy animals /37/. Other investigators found it only in the brain of animals in pathological states. Foreign scientists have shown that the glycogen content in the brain remained constant.

We found that brain glycogen is not an inert substance but is subject to continuous transformations. The rapid metabolism of glycogen apparently is responsible for the fact that under normal conditions it does not accumulate in significant amounts in brain tissue. For this reason numerous investigators did not succeed in detecting it in the cerebral cortex and in the cerebellum.

The inaccuracy of the conception of the unchanging glycogen content in the brain can be seen from the results of our studies on the carbohydrate metabolism of the brain in various functional states. Thus, when the central nervous system is excited to the degree when convulsions result (through the application of an electric current or cardiazol administration) the activity of amylase increases while the total glycogen content decreases. During anesthesia, on the other hand, amylase activity decreases and the glycogen content of the brain increases /38/.

Hence, brain enzymes may display different activity in various functional states of the central nervous system. Glycogen is subject to continuous transformations in the brain and its content either decreases or increases. The ratio between free and bound glycogen also changes. Thus, for example, during convulsions the total glycogen content decreases at the expense of free glycogen, while the fraction of bound glycogen even increases somewhat. At present it is difficult to state whether the two fractions of glycogen have any physiological significance. The available data on this subject are contradictory.

We determined the contents of both glycogen fractions while studying the effects of hypoxia on the carbohydrate metabolism of the brain. The data obtained did not allow us to reach a final conclusion on the roles of free and bound glycogen. We studied the various glycogen fractions and determined their contents in the brains of rabbit embryos and of young rabbits /39/. We showed that in the brains of embryos and newborn rabbits during the first few days after birth the free form of glycogen prevails. Subsequently, with the development of the animal brain, the content of free glycogen decreases and the fraction of bound glycogen increases.

In the brain of adult animals the latter fraction prevails. This apparently indicates that bound glycogen may be better utilized in metabolic processes. However, this problem warrants further study.

The central nervous system is extremely sensitive to oxygen deficiency. Even a slight decrease in the oxygen content of the air may lead to a disturbance of nervous activity.

Since carbohydrates serve as a main source of energy, much research has been devoted to carbohydrate metabolism during hypoxia. The studies were conducted mainly on blood and on a number of other tissues (muscle and liver).

The available data on carbohydrate metabolism in the brain during hypoxia are few and contradictory. However, they indicate that there is a disturbance of carbohydrate metabolism during oxygen deficiency. Since the picture is not complete, we decided to study this problem in greater detail.

We studied the effects of hypoxia on the rate of glycolysis, on the contents of preformed lactic acid and of glycogen (both free and bound), and on the activities of various enzymes. These enzymes included hexokinase, which catalyzes the phosphorylation of glucose — the first step in glycolysis, and phosphorylase and amylase, which catalyze the hydrolysis and synthesis of polysaccharides.

The experiments were conducted on rats and rabbits. Rabbits were kept in a pressure chamber at an altitude of 7000-10,000 meters for 4-7 hours. Rats were kept in the pressure chamber for 4 hours at an altitude of 6000-8000 meters. Rabbits endured hypoxia better than did the rats.

The experiments on rabbits showed (Figure 1) that disturbances in the carbohydrate metabolism of the brain manifest themselves first in the accumulation of preformed lactic acid. This, apparently, is due to the decreased rate of the aerobic oxidation of lactic acid.

In brain hypoxia glycolytic processes prevail over respiration. Since we observed in our experiments a tendency toward a lower rate of glycolysis, it must be assumed that rabbits, upon prolonged stay at high altitudes, manifest disturbances in glycolysis, and possibly irreversible ones.

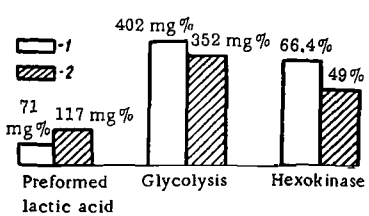


FIGURE 1. Carbohydrate metabolism in the brain of rabbits during hypoxia

1 — Control; 2 — Hypoxia

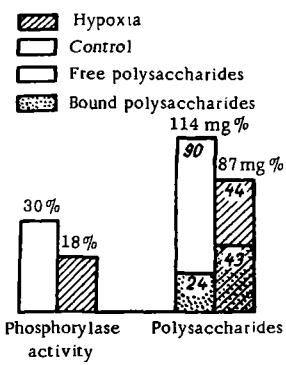


FIGURE 2. Polysaccharide content and synthetic activity of phosphorylase in the brain of rats during hypoxia

Studies of hexokinase showed that its activity decreased during hypoxia. Hence, hypoxia of the brain leads to disturbances even in the first stage of glucose transformation in the brain.

Our observations showed that the total glycogen content of the brains of rabbits under hypoxic conditions increases by about 60% but that the distribution between free and bound glycogen does not change. The activity of the phosphorylase catalyzing the synthesis of polysaccharides increases, which indicates that favorable conditions are created for the synthesis of polysaccharides. The activity of enzymes participating in glycogen hydrolysis (phosphorylase and amylase) is not affected.

Our experiments confirmed the presence of disturbances in carbohydrate metabolism during hypoxia. Utilization of glucose is affected as judged by the lower activity of hexokinase. The unutilized glucose is used for the synthesis of polysaccharides.

The experiments on rats gave unexpected results (Figure 2). When the glycolytic activity of rats and rabbits decreased, and there was no accumulation of lactic acid during tissue autolysis in the absence of substrates (which indicates a complete utilization of endogenous carbohydrate substrates), the glycogen content did not increase, but rather decreased by about 30% as compared with control animals. The content of bound glycogen increased and that of free glycogen decreased. The synthetic activity of phosphorylase also decreased, while its phosphorolytic capacity was somewhat enhanced.

These differences in the degree of disturbance in the carbohydrate metabolism of rabbits and rats during hypoxia are due, apparently, to the different sensitivities of these animals to hypoxia. To obtain a clear picture of the disturbances in the carbohydrate metabolism of animals during hypoxia, further studies involving different animals are needed. It is necessary to obtain enough data to enable one to direct metabolic activity in the desired paths.

One of the main problems in the biochemistry of the nervous system is that of the correlation between the functional state of the central nervous system and its metabolic processes.

Metabolic studies on the chronic functional weakening of the cerebral cortex are of great importance since nothing is known about the changes in metabolic processes caused by the functional weakening of the nervous system.

We approached [/40/] this problem by studying the rate of oxidation in the brain and liver of rats with a functional weakening of the central nervous system caused by keeping the animals for several days in electrode cells, according to the method of Petrova /41/.

Our studies showed that rats with a functional weakening of the central nervous system displayed a slight decrease in the rate of respiration both in the brain and in the liver, thus showing the dependence of liver metabolism on the functional state of the brain. Similar results were obtained in metabolic studies on the nucleic acids in the brain and liver when the functional state of the brain was altered /42/. In anesthetized animals, with a resulting inhibition of the higher nervous activity, nucleic acid metabolism was altered both in the brain and in the liver, as judged from nucleic acid content and the nuclease activity.

Partial hepatectomy caused alterations in the nucleic acid metabolism of the liver and also of the brain. The above data confirm the fact that

metabolic activity in the various organs is under the control of the cerebral cortex and becomes altered during the functional changes of the latter. On the other hand, the cortex is affected by the various stimuli appearing in the different organs.

Metabolic disturbances in various organs and tissues of a living organism are not always accompanied by similar changes in brain metabolism. Thus, for example, our studies showed that disturbances in carbohydrate metabolism appearing after the administration of phlorizin and adrenaline, accompanied by changes in creatine metabolism in muscle and excretion of creatine in urine, do not affect creatine metabolism in the brain (see Epstein /43/). Likewise, injection of guanidine, which raises the creatine level in muscle and increases creatine excretion in urine, does not affect creatine content in the brain (Feinschmidt, /43/).

Pavlov repeatedly stressed that the main processes characterizing higher nervous system activity are those of excitation and inhibition and that our understanding of them depends on our knowledge of the physical-chemical processes taking place in the nervous system. Thus the importance of the biochemical decoding of these main functional states of nerve tissue is apparent. Considering this as the central problem in the study of the biochemistry of the brain, we decided to investigate some metabolic processes in the brains of animals during inhibition or excitation of the higher nervous activity.

We limited our investigations /44/ to nucleic acid, carbohydrate, and ATP metabolism. Nucleic acids play an extremely important role in protein metabolism. Carbohydrates serve as the main source of energy for brain activity. The adenosine triphosphate present in nervous tissue probably has the same function as in muscle — the linking of energy-yielding processes with specific synthetic reactions in the brain.

In the first stage of our study we determined the contents of ribonucleic and deoxyribonucleic acids and the activity of deoxyribonuclease. Brain tissue, as all other tissues, contains both ribonucleic and deoxyribonucleic acids, the ratio of the former to the latter being about 3. Ribonucleic acid plays an important role in processes connected with protein synthesis. Deoxyribonuclease catalyzes the degradation of deoxyribonucleic acid.

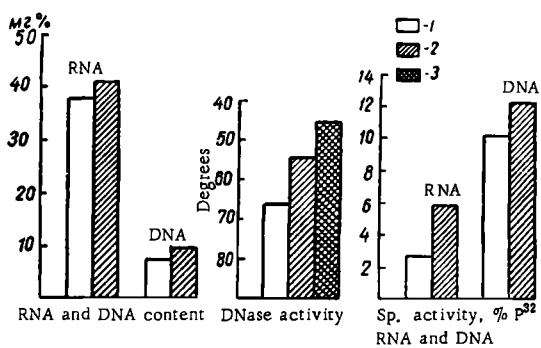


FIGURE 3. Nucleic acid content, deoxyribonuclease activity, and turnover of nucleic acid phosphorus during narcotic sleep

1 — Control; 2 — 4 hr sleep; 3 — 6 hr sleep

Our studies on nucleic acid metabolism in anesthetized animals (Figure 3) show that their deoxyribonuclease activity is considerably increased and that the increase is proportional to the duration of anesthesia. However, only slight changes were observed in the ribonucleic and deoxyribonucleic acid contents. The fact that a considerable change in the enzymatic activity of deoxyribonuclease results in only slight changes in nucleic acid content indicates that during prolonged anesthesia an active turnover of nucleic acids takes place, and thus synthetic processes prevail over those of degradation.

This conclusion has been confirmed by studies with P^{32} on the turnover rate of nucleic acids. The specific activity of RNA phosphorus in anesthetized animals was considerably higher than in control animals. Similar results, but less pronounced, were found for deoxyribonucleic acid.

Our studies on carbohydrate metabolism in anesthetized animals showed that their content of preformed lactic acid was lower than in control animals. Their glycolytic activity remained virtually unchanged whereas the glycogen level (both free and bound) increased.

These data indicate that during anesthesia there is a lower expenditure of carbohydrates. This conclusion was confirmed by our studies of the enzymes involved in carbohydrate metabolism. Thus, for example, hexokinase activity decreased while that of amylase and phosphorylase remained unchanged.

The level of adenosine triphosphate increased in anesthetized animals, (Figure 4) probably due to a lower ATP expenditure.

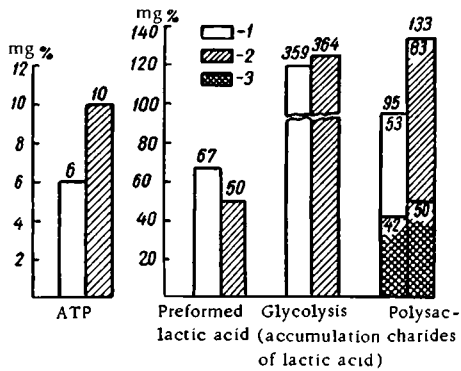


FIGURE 4. Metabolism of carbohydrates and adenosine triphosphate in the brain of animals anesthetized for four hours

1 — Polysaccharide control — free polysaccharide in control; 2 — Anesthesia, free polysaccharide; 3 — Anesthesia, bound polysaccharide

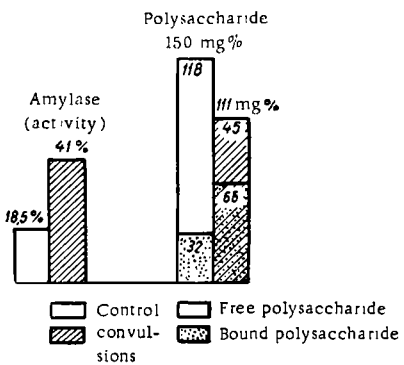


FIGURE 5. Amylase activity and polysaccharide content in the brain during prolonged excitation, loading to convulsion

According to Pavlov inhibition is a process which protects nerve cells from destruction and facilitates their regeneration. Narcotic sleep may serve as a model for protective inhibition. Pavlov's studies on protective inhibition have shown that during sleep the brain is far from being inactive. Biochemical processes are not arrested or even diminished but are directed mainly towards the recovery of brain activity.

Our data constitute the first attempt to interpret the phenomenon of protective inhibition in metabolic terms. These data show that during narcotic sleep metabolic activity is not arrested or even diminished; on the contrary, the activity of some enzymes is enhanced. The nucleic acid content does not decrease, carbohydrate expenditure is lowered, and the adenosine triphosphate level increases. It can therefore be concluded that during anesthesia synthetic processes prevail over those of degradation and hydrolysis, facilitating the restoration of brain activity.

Prolonged excitation, leading to convulsions, causes a decrease in the levels of polysaccharides and adenosine triphosphate, with a concomitant increase in the activity of the enzymes which catalyze the hydrolysis of these substances (Figure 5). Similar data were obtained by Minaev and Vladimirova.

To avoid exhaustion of the nervous system we induced the state of excitation by a single administration of a small dose of pervitin which, like phenamine, is widely used in medicine as a stimulant of higher nervous activity. We also found cardiazol useful for this purpose. When excitation was induced with these drugs in rabbits, their mobility increased, their reflectory excitability was heightened, and they tapped with their posterior extremities.

The effect of pervitin on brain metabolism was different from that of cardiazol /45/. During the excitation of higher nervous activity induced by pervitin the level of nucleic acid in the brain remained almost unchanged. The turnover of ribonucleic acid remained unaltered and that of deoxyribonucleic acid decreased somewhat. It has therefore been concluded that under these conditions there is almost no change in nucleic acid metabolism (Figure 6).

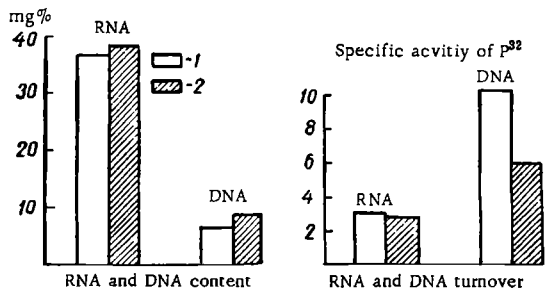


FIGURE 6. Nucleic acid and P^{32} turnover in the brain after administration of pervitin

1 — Control; 2 — Pervitin

Under the influence of pervitin the level of preformed lactic acid decreased as compared with control animals and those which received cardiazol. The rate of anaerobic glycolysis in the brain increased. This indicates that administration of pervitin creates conditions favoring enhanced carbohydrate activity concomitant with enhanced ATP synthesis manifest in the increased level of ATP in the brain (Figure 7). Adenosine triphosphate is an active biochemical compound, the accumulation of which enhances the activity of cerebral cortex. These data indicate that the

stimulatory activity of pervitin on higher nervous activity is due to the enhanced metabolic activity of the brain.

The state of excitation caused by cardiazol is characterized by an increased level of preformed lactic acid. Glycolysis is much less affected than with pervitin and the adenosine triphosphate level remains almost unchanged.

The above data show the effect of various stimulating compounds, such as pervitin and cardiazol, on the brain. Our data show for the first time the reasons for the different physiological effects of the various stimulating drugs. They also confirm the teaching of Pavlov.

The aim of our research was to study the metabolic processes of the brain in its various functional states. We have attempted to elucidate the metabolic role in any given function of the various brain regions, and particularly of the cerebral cortex, and to determine the control exerted by the environment.

We have studied the chemical structure, the protein content, and the metabolic processes (protein and carbohydrate metabolism) of functionally different regions of the brain. We have investigated metabolic changes by determining the changes in chemical structure and in the content of intermediary and end products of metabolic processes. We have determined the activities of various enzymes participating in the biochemical reactions studied. These studies were carried out in various functional states of the brain. We have also taken the first steps along the difficult road of controlling and directing metabolic processes in the brain. The ultimate goal of biochemical research is to elucidate the factors which control the metabolism of living organisms, and of man. As Pavlov said "Only he who is capable of restoring to norm its disturbed processes, can say that he understands life" /46/.

A deep understanding of reality and an ability to influence life are the main features of the philosophy of dialectic materialism. This is also the direction of Soviet biology, including Soviet physiology and biochemistry, as reflected by Michurin: "We cannot wait for favors from nature, we have to take them — this is our task."

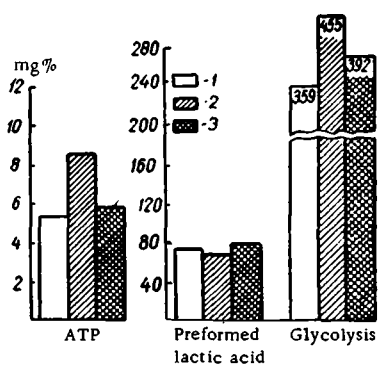


FIGURE 7. Changes in carbohydrate metabolism and in ATP content during excitation caused by pervitin and cardiazol

1 — Control; 2 — Pervitin; 3 — Cardiazol

In our experiments, we have also attempted to elucidate the biochemical processes in narcotic sleep as a model of protective inhibition and to find the biochemical reasons for the various physiological effects of the different stimulating drugs. These results undoubtedly will be of some use to physiologists and psychiatrists.

We have also shown that the brain affects the metabolism of other tissues and that stimuli appearing in various organs affect cortical metabolism.

The results of our studies, though not free from shortcomings, are necessary for the understanding of the regulatory role of the central nervous system in metabolism, and for the biochemical unraveling of metabolic pathways

and processes of adaptation with the aid of which higher regions of the central nervous system regulate metabolic processes underlying the various physiological functions of individual organs and of the living organism as a whole.

To solve this problem further studies are needed. These studies should be carried out by biochemists in collaboration with physiologists and physicians. By studying the effect of the nervous system on metabolism (these problems are now being studied in various biochemical laboratories of the USSR), the chemical nature of living processes, and the metabolic pathways, so as to enable us to control them, the Soviet biochemists, armed with the philosophy of Marxism and Leninism, will participate in the building of Communism.

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*PROBLEMS IN THE BIOCHEMISTRY OF THE NERVOUS SYSTEM**

The works of I. M. Sechenov have laid a firm foundation for the development of physiology and physiological chemistry in the Soviet Union. We owe to Sechenov and Pavlov the materialistic scientific conception without which biological sciences could not develop.

Sechenov and Pavlov developed the concept of the unity of an organism with its environment and showed that the functions of a living organism are regulated by the nervous system. In 1861 Sechenov wrote: "It is impossible to think of an organism without the environment which supports its existence. Therefore, the scientific concept of an organism should include its environment." Any process in the whole organism, be it tissue metabolism or the most complex forms of activity, such as psychic processes, is directed by the cerebral cortex.

The development of Soviet physiology, its problems and directions, has been influenced by Sechenov's work, "Reflexes of the Brain," which brought him fame. This work had a great influence on the development of physiological chemistry, now called biochemistry. It may also be said that this work laid the foundation for the research on the biochemistry of the nervous system.

Studies on the biochemistry of the central nervous system, namely studies on the chemical composition of various brain sections, were started already in the nineteenth century. Among the prominent works which appeared at that time were those of Russian scientists, such as R. Ya. Danilevskii, one of the first Russian biochemists. Danilevskii understood the importance of proteins in the activity of the nervous system and devoted much of his study to brain proteins. He also developed a method for their fractionation and used it in his studies on the protein composition of the brain. It should be pointed out that Danilevskii did not confine himself to the study of the protein compositions of the brain as a unit but, with his collaborators, carried out comparative biochemical investigations on the protein composition of the brain in its various functional states. He also investigated the biochemical processes of the brains of various phylogenetically different animals.

According to Danilevskii (1891) it is necessary to study the biochemistry of brain in various functional states. He wrote: "There is not a single tissue, apart from brain tissue, where the interdependence of chemical composition and function is of such deep biological interest and at the same time is so obscure and difficult to understand." The work of Danilevskii was continued by other Russian scientists, such as N. Shkarin, A. Lents and B. Slovtsov.

* Lecture read at the All-Union Conference of Biochemistry of the Nervous System held in Kiev on 15 December, 1953 (see collection: Biochemistry on the Nervous System Kiev, pp. 7-24. 1954.)

The pioneer in the area of brain lipids was also a Russian scientist, R. Petrovskii, who in the 19th century determined the lipid content in cattle brain. He was also one of the first to determine the protein content in the gray and white matter of the brain.

Rapid progress in scientific activity in our country after the Socialist Revolution led to comprehensive studies in the biochemistry of the nervous system. From 1925 on, studies on the biochemistry of the brain have been conducted in the Ukrainian Biochemical Institute (today the Institute of Biochemistry of the Academy of Sciences of the Ukrainian SSR). Later, such studies were also begun in Leningrad. In 1937 the works of E.A. Vladimirova of the Institute of Physiology were published. Still later, E.M. Kreps and his collaborators began to study the chemical processes of the brain during ontogenetic development. The first results of these studies were published in 1946. In the thirties, S.Ya. Kaplanskii published his results on the amino acid composition of brain proteins and on the composition of brain phosphorus compounds.

Before the second world war, Koshtoyants began the study of acetylcholine and its conversions in the brain. Later, in Gorki, G.Ya. Gorodisskaya and her collaborators studied brain nitrogen metabolism. These studies were a continuation of the work which she began in the Ukrainian Biochemical Institute.

Several aspects of brain biochemistry were studied in the different laboratories. Among these were the chemical aspect (the chemical composition of brain sections), the dynamic aspect (enzymatic studies), and the functional aspect (the study of brain metabolism in various functional states).

As I have already mentioned, the Russian scientists of the 19th century already used the functional approach to the study of the biochemistry of the central nervous system. In the Ukrainian Biochemical Institute, now the Institute of Biochemistry of the Academy of Sciences of the Ukrainian SSR, research on the biochemistry of the nervous system, and especially of the brain, was also mainly of a functional nature, in that the main emphasis was placed on the functional states of the brain. However, dynamic aspects were also studied.

In 1947, in summarizing our research on the biochemistry of the brain, I said that these studies aimed at clarifying such problems as whether the functionally various brain sections differ in chemical composition, and whether there are any alterations in their composition in the process of functional development. These studies were conducted in order to determine whether there is any correlation between the characteristic properties and activities of the various brain sections and their chemical composition. We attempted to show how metabolic processes are altered in the different brain sections during changes in their activity under the influence of various factors.

Our first area of study was the proteins and their metabolism in brain tissue. We investigated the chemical composition of various brain sections of animals of different phylogenetic development and of various regions of the central nervous system which were histologically similar or closely related but differed functionally and phylogenetically. We also studied the chemical composition of the spinal cord ganglia, of sections of the autonomous nervous system, of various parts of the peripheral nervous system, and of brain sections of various phylogenetic and ontogenetic development.

We showed that the phylogenetically youngest and functionally most complex sections of the nervous system are the richest in protein material, which indicates that proteins play an important role in the central nervous system. We also showed that the various brain regions differ in chemical composition long before birth, and that their chemical composition changes during embryonic and postembryonic development in correlation with their functional development.

Our studies on brain nitrogen metabolism during various conditions, such as vitamin deficiency, starvation, and upon administration of pharmacological drugs, showed that changes in the functional state of the brain are closely related to changes in metabolic processes, which are dependent upon environmental conditions.

We found also that the gray and white matter of the brain differ from each other not only in the quantity of protein which they contain, but also in the nature of their proteins; that is, they contain different protein fractions.

We have found that the transition of the cerebral cortex from a state of relative rest to that of increased activity is accompanied by an enhancement in nitrogen metabolism. Finally, it was shown at the Institute of Biochemistry that the cerebral cortex displays not only morphological and functional topography but also chemical topography.

Much of our research has been devoted to carbohydrate metabolism. We showed that in brain carbohydrate metabolism intermediate phosphorylated compounds are formed similar to those found in muscle glycolysis, and that the rate of glycolysis may change during ontogenetic development and differs in the various functionally different brain sections.

We have investigated the enzymes associated with carbohydrate metabolism in the brain and found a very active amylase, which catalyzes the hydrolysis of polysaccharides. We also ascertained the presence of the enzymes hexokinase, phosphoglucomutase, phosphatase, aldolase and adenosine triphosphatase and studied their properties. We found that the functionally complex brain regions are characterized by a higher enzymatic activity.

In recent years an ever increasing number of institutions and scientists have become engaged in the study of the biochemistry of the nervous system. Continued efforts in this direction will bring nearer the time when, as Pavlov said, ".. an accurate and complete knowledge of our highest organ, the brain, will be our genuine contribution and, hence, the basic source of lasting human happiness."

The rapid progress in the field of the biochemistry of the nervous system may be clearly seen by comparing the programs of two scientific conferences on the biochemistry of the nervous system. The first was held in January 1938 in Kiev and was sponsored by our Institute. The second is the present conference on the biochemistry of the nervous system.

At the first conference only eleven lectures were presented and read. Five of them were from the Institute of Biochemistry of the Academy of Sciences of the Ukrainian SSR, two from the Department of Biochemistry of the Gorki Medical School, headed by G. Ya. Gorodisskaya, and the remaining were read by Professor S. Ya. Kaplanskii and others. Thus, at that time works on the biochemistry of the nervous system comprised a very small part of the field of biochemistry.

Now consider the program of the conference of 1953 where 24 lectures were read, while the majority of those presented could not be read due to the lack of time. The program shows that at present aspects of the biochemistry of the nervous system are being studied in a large number of scientific institutions of Moscow, Leningrad, Kiev, Kharkov, Tbilisi, Minsk, Odessa, Vitebsk, and other cities.

The Pavlov sessions have indicated that further research in the field of biochemistry should stress mainly its functional aspects. Academician K. M. Bykov discussed this topic briefly in his lecture, while Academician V. A. Engel'gardt* and Professor G. E. Vladimirov elaborated upon in it greater detail. The need for the development of functional biochemistry was stressed by me in one of the sessions of the Academy of Sciences of the Ukrainian SSR when I said that "the chief task which faces Soviet biochemistry, which ought to represent Pavlovian biochemistry, is the study of the metabolism of the entire living organism in the various functional states and in various environments. It is necessary to illustrate the interrelations between specific functions of organs and systems and the details of their metabolism, especially in nerve, muscle, and glandular tissue. Also of great importance is the study of the mechanism of metabolic regulation by the nervous system."

Pavlov has repeatedly stressed that the main processes which characterize higher nervous activity are those of excitation and inhibition. In this respect one of the most important problems of Soviet biochemistry is the study of brain metabolism during excitation and inhibition in order to understand the biochemical principles underlying the physiological processes of the central nervous system. Such studies should reveal the regulatory mechanisms of various metabolic processes and bring us closer to our ultimate goal — the possibility of actively influencing metabolic processes.

Based upon these considerations, we at the Institute of Biochemistry of the Academy of Sciences of the Ukrainian SSR have for the past three years studied carbohydrate metabolism of the brain with particular emphasis upon excitation and inhibition of nervous activity.

In these experiments various approaches are possible. The state of excitation or of inhibition of the central nervous system may be caused experimentally by different means. Various conditioned and unconditioned stimuli of varying strength may be used, either natural or purely experimental, by whose means a variety of physical responses may be elicited from the organism. In addition, a wide variety of drugs may be employed from the vast store of medicinal preparations.

It was clear to us that metabolic processes may vary according to the functional state of the nervous system. The first step was to establish some common features of brain metabolism during excitation or inhibition and for this purpose we made use of various drugs. Our next step was to use both unconditioned and conditioned stimuli.

The aim of our investigations was to study the nature of the metabolism of nucleic acids, carbohydrates, and adenosine triphosphate (ATP). Nucleic acids, first studied by Danilevskii in the 19th century, play an extremely important role in tissue protein metabolism. Carbohydrates are the main source of energy for brain activity. ATP, in muscle as well as nerve tissue, is apparently the connecting link between energy-yielding processes

* [Also referred to in the literature as Engelhardt.]

and those metabolic processes which determine the biochemical specificity of the nervous system.

Nucleic acid metabolism was studied by the separate determinations of the contents of ribonucleic and deoxyribonucleic acids and the activity of deoxyribonuclease. Brain tissue, like all other tissues, contains both nucleic acids, the content of ribonucleic acid being about three times that of deoxyribonucleic acid. It is known that ribonucleic acid plays an important role in protein synthesis. Deoxyribonuclease is responsible for the first stage of deoxyribonucleic acid metabolism, namely, its degradation.

Carbohydrate metabolism was studied by the separate determinations of the contents of preformed lactic acid and polysaccharides (both free and bound), glycolytic activity, and the activity of the enzymes hexokinase, amylase and phosphorylase.

To obtain a better understanding of the role of a phosphorylated compounds in nerve tissue in various functional states we studied, in addition to ATP, acid-soluble fractions of phosphorus, inorganic phosphorus, and phospholipids (both saturated and unsaturated).

Experiments were carried out on rabbits, dogs, and rats. In studies of this kind, when it is necessary to determine the composition and metabolic processes of the brain in a specific functional state, the method of sacrificing the animal is of great importance. Therefore, in the first stage of our work, the animals were quickly decapitated, whereupon the cerebral hemispheres were extracted and frozen in liquid air. Another method consists in directly freezing the severed head in liquid air and then extracting the hemispheres. This method is useful in experiments with small animals, since with large animals some time elapses between decapitation and freezing of the brain which, although not very long, may be sufficient for various changes to occur in the content of labile phosphorylated compounds.

During decapitation there often appear convulsions and other disturbances which may affect the labile phosphorylated compounds and distort the characteristic appearance of the given functional state. To avoid this, hexenal may be injected prior to decapitation. One ml of a 10% solution of hexenal given intravenously leads to immediate death due to paralysis of the respiratory center. This method has recently been used by us.

It is also possible to immerse the entire animal in liquid air, a method which E. A. Vladimirova uses with rats, utilizing for this purpose specially constructed cages. However, this method undoubtedly leads to a strong, though shortlived, stimulation of skin receptors, and these stimuli may be conducted to the brain before it freezes and bring about metabolic changes which can distort the functional state under study. All this shows that it is necessary to select carefully the method of sacrificing the animal, especially when the enzymatic activity of the brain is concerned.

The state of excitation was induced by the administration of pervitin which, like phenamine, is widely used as a stimulant of higher nervous activity. Cardiazol was also found useful for this purpose.

We found /1, 2, 3/ that strong excitation, leading to convulsions (injection of a large dose of cardiazol or stimulation with an electric current) and exhaustion of nervous system, results in a decrease in the ATP and polysaccharide contents of the brain.

In other experiments, more closely approximating physiological conditions, excitation was induced by a single administration of a small dose of pervitin (5 to 7 mg per kg of body weight) or of cardiozol (50 to 70 mg per kg of body weight) four hours before sacrificing the animal. We found that the various stimulatory compounds elicit different responses in brain metabolism /1/.

Animals treated with pervitin showed a higher rate of anaerobic glycolysis than did control animals, or those treated with cardiazol /4/. Excitation induced by pervitin resulted in an increase in ATP content, while that induced by cardiazol had no such effect. It is clear that pervitin creates conditions for the enhanced activity of oxidative processes, accompanied by an increased synthesis of ATP. This is not the case with cardiazol. No change in nucleic acid content was observed with the administration of pervitin.

The best method for studying the complex and biochemical processes which characterize the activity of the central nervous system is that making use of radioactive isotopes. This method enables one to study not only the quantitative changes of a compound in a given tissue but also its rate of incorporation, turnover, synthesis, and degradation. In view of this we employed radioactive phosphorus, P^{32}, in our study of nucleic acid metabolism. Radioactive phosphorus was administered intravenously together with pervitin.

In order to obtain information about the turnover rate of nucleic acids during excitation we determined their specific activity (P^{32}). Since our studies showed that the specific activity remains unchanged, it may be concluded that no marked changes in nucleic acid metabolism occurs /5/ during pervitin induced excitation.

Our studies showed that the various stimulatory substances affect brain metabolism in different ways, depending upon their different physiological effects. Pervitin-induced excitation evokes metabolic changes characterized by an increased metabolic rate and an accumulation of ATP, which increases the activity of the cerebral cortex. Thus our data explain the various physiological effects of the different stimulatory drugs.

Considering the fact that the nature of stimulation may differ, depending upon the drug used and upon the duration of its action on the nervous system, we found it indispensable to study the metabolism of phosphorus-containing compounds at various periods after the administration of pervitin or cardiazol (after 1, 2 and 4 hr).

Our results have shown /6/ that during the first hour after the administration of pervitin the ATP level of the brain at first decreases and then gradually increases, reaching the normal level after two hours. Four hours after the administration of pervitin the ATP level is considerably higher than that in control animals. These data confirm the results of our previous studies in which the ATP content of the brain was determined four hours after the administration of pervitin. They also show that ATP metabolism differs at various time intervals after the administration of pervitin.

Changes in inorganic phosphorus show an opposite direction from that of ATP. During the first two hours the level of inorganic phosphorus increases somewhat but then decreases so that after four hours it is lower in control animals.

The results obtained during cardiazol-induced excitation were quite different from those obtained with pervitin. During the first hour after the administration of cardiazol the ATP level increased, then gradually decreased till after two and especially after four hours, its content was much lower than that in control animals.

Here again, changes in inorganic phosphorus in control animals showed an opposite direction. During the first two hours the level of inorganic phosphorus decreased then gradually increased, surpassing that of control animals after four hours.

These experiments, corroborating the data obtained earlier, showed once again that pervitin and cardiazol, having different physiological activities, affect brain ATP metabolism in different ways.

We also studied ATP metabolism with the aid of radioactive phosphorus. Animals were sacrificed one, two, and four hours after the administration of pervitin or cardiazol, given concomitantly with radioactive phosphorus. The relative specific activity of ATP phosphorus was then determined as the ratio of the specific activity of ATP to that of inorganic phosphorus.

These studies showed that the relatively specific activity of ATP phosphorus in excitation induced by pervitin differed from that induced by cardiazol. During pervitin-induced excitation the relative specific activity of ATP phosphorus increased, as compared with control animals, in the course of the first four hours after the administration of pervitin. This indicates that ATP metabolism is enhanced during the interval from one to four hours after the injection of pervitin.

The relative specific activity of ATP phosphorus during cardiazol-induced excitation was diminished during the first three hours and began to approach control values only towards the fourth hour. This indicates a decreased rate of ATP phosphorus turnover. Hence, pervitin is more effective than cardiazol in increasing the metabolic activity of ATP.

Thus, the data obtained with the aid of radioactive phosphorus indicate that pervitin and cardiazol affect brain tissue metabolism, and particularly ATP metabolism, in different ways. These biochemical differences are caused by the different physiological effects of the two drugs; pervitin stimulates the central nervous system and increases its activity, whereas cardiazol stimulates cerebral cortex but does not increase its activity.

Studies on phospholipids during pervitin-induced excitation showed that the total phospholipid content, as well as that of the saturated and the unsaturated fractions, remains unchanged during the four hour interval after the administration of pervitin /7/.

However, with the use of radioactive phosphorus it was possible to show that the incorporation of phosphorus into both phospholipid fractions during pervitin induced excitation differs from that observed in control animals. The relative specific activity of the phosphorus of saturated phospholipids increased markedly during the first hour and then began to fall, approaching control values during the third hour, and being lower than the control by the fourth hour. The specific activity of unsaturated phospholipids was somewhat higher than the control during the first two hours and then decreased below the control values. These data show that alterations in phospholipid metabolism accompany changes in the functional state of the nervous system and that phospholipids are metabolically active compounds.

In further stages of our investigations of brain metabolism during excitation of the nervous system, we studied the condition of hyperexcitation accompanied by the impairment of nervous activity. Chronic hyperexcitation was induced by an electric current or by disturbances in physiologic sleep. In the first method the animals were kept in special electrode caves (according to the method of Petrova) and an electric current of 25 to 40 volts was applied daily (10 applications, each lasting one minute, with a two-minute interval between applications) for a period of one to one-and-a-half months. The rats were studied thirty minutes after the last application of electric current. In the second method, sleep was disturbed for three successive days by placing the rats in rotating drums (30-second rotations followed by five-minute intervals).

The results showed that during forced chronic insomnia the glycolytic activity of the brain was somewhat lower than in control animals. The glycogen content remained unchanged, while the ATP content decreased. Hyperexcitation induced by an electric current caused a decrease in ATP content.

We also studied ATP phosphorus metabolism during forced insomnia with the aid of radioactive phosphorus and found that the specific activity of phosphorus was lowered. It seems that under the conditions employed hyperexcitation led to exhaustion of the nervous system, which in turn brought about a decrease in the metabolic rate of labile phosphorylated compounds.

Our investigations also included the effect of inhibitory drugs on brain metabolism. For this purpose we employed the drug medinal in such doses as to closely approximate natural sleep. We took into consideration the fact that natural sleep evolves from the previous activity of the animal while pharmacological drugs induce sleep without such previous activity and its effects upon the organism. Therefore, the metabolic characteristics of narcotic sleep may not be completely analogous to those of natural sleep.

Studies on nucleic acid metabolism in rats during prolonged narcotic sleep have shown that there is a significant increase in the activity of deoxyribonuclease, which is proportional to the duration of sleep. At that time there are only slight changes in the contents of ribonucleic and deoxyribonucleic acids. Comparison of the changes in deoxyribonuclease activity with the changes in nucleic acid content indicates that during narcotic sleep there is a high nucleic acid turnover and that synthesis prevails over degradation. This follows from the fact that in spite of the considerable increase in deoxyribonuclease activity the content of nucleic acids remains unchanged.

Studies on carbohydrate metabolism during narcotic sleep showed that the rate of glycolysis in rabbit brains was quite high, resembling that of control animals. The polysaccharide content increased, and hexokinase activity decreased slightly /8/, while the activities of amylase and phosphorylase remained unchanged. This indicates that the rate of carbohydrate metabolism remains quite high during narcotic sleep /1, 2/. The content of ATP also increased.

According to Pavlov, inhibition is a process which protects nerve cells against exhaustion and facilitates the restoration of their activity. Narcotic sleep may be regarded as a model of protective inhibition. According to Pavlov's theory of protective inhibition, the condition of sleep

does not mean that the brain is inactive and that its metabolic processes are arrested. Such processes may not even be diminished. They are directed towards the restoration of brain activity.

The data obtained by us represent an attempt to shed some light on the biochemical aspect of sleep as a mechanism of protective inhibition. They show that during narcotic sleep metabolic activity is neither arrested nor diminished. Quite to the contrary, the activity of some enzymes and the content of ATP increase, while carbohydrate and nucleic acid metabolism remains on a high level. Anabolic processes prevail over those of catabolism facilitating the restoration of brain activity.

The results of some of our studies in the functional biochemistry of the brain confirm the physiological concepts of Pavlov on the nature of nerve tissue processes under the physiological conditions studied and indicate, I think, that the direction of our studies in the biochemical definition of the main functional states of the central nervous system is correct.

In other studies, we approached the problem of the interrelation between organic functions and the activity of the higher regions of the central nervous system. We studied the changes in the metabolism of the peripheral organs (liver) during functional changes of the cerebral cortex and the effect of these organs on brain metabolism /9/. We also studied the effect of stimulating various receptor fields of one half of the cerebral cortex and found that it resulted in a fairly stable assymetry of metabolism in the two cerebral hemispheres /10/.

The papers published in this book indicate that numerous institutes in the Soviet Union are at present engaged in studies on the biochemistry of the nervous system, and particularly on the brain.

Metabolic studies of the brain in its various functional states are presently being conducted in a number of scientific institutes of our country. Scientists such as G.E. Vladimirov and E.A. Vladimirova, like us, are engaged in the study of brain metabolism during excitation and inhibition of nervous activity. E.M. Kreps is conducting comparative biochemical studies on brain metabolism. Various other features of brain metabolism are being studied by V.A. Engel'gardt, P.A. Kometiani, V.C. Shapot, and A.M. Kuzin. There is no doubt that collective work on this important problem, together with creative criticism and discussion of the results and the methods of investigation, will in a short time enable Soviet biochemists to define biochemically the main functional states of brain.

There is no doubt that in biochemical studies of the processes which characterize nervous activity special attention should be paid to brain proteins. These are studied in some laboratories of the Soviet Union. However, not enough has been done in this area. Studies on protein metabolism should be widened in scope and special attention should be paid to protein complexes of nerve tissue.

Much attention is devoted in the USSR to the study of energy-yielding reactions in the brain. (A.V. Palladin and collaborators, G.E. Vladimirov and collaborators, and others). It is possible that studies in this direction will yield the most reliable information on the problem with which we are concerned.

Another important field in the biochemistry of the nervous system is that of nervous regulation of mediator and hormone metabolism. This problem

is studied by such Soviet scientists as Kh.S. Koshtoyants, A.M. Utevskii, D.E. Alpern, and P.A. Kometiani. Physicochemical reactions during nervous activity are studied by G.M. Frank.

It should be remembered that research in the functional biochemistry of the nervous system cannot achieve spectacular results without clinical studies — without the collaboration of pathologists, neurosurgeons, and psychiatrists (A.B. Speranskii, V.P. Protopopov). Only with their aid can we obtain data about the biochemistry of the human brain in its various pathological states.

All Soviet biochemists agree that the main effort of our studies should be in the direction of functional biochemistry. This, however, does not negate the value of studying the various aspects of dynamic biochemistry in animal tissue (enzymology, etc.). Although functional biochemistry does not include studies on the chemical composition of nerve tissue and its structural elements, such studies should not be excluded from the work of Soviet biochemists. Likewise, works in the areas of dynamic and descriptive biochemistry should be utilized towards the solution of problem in functional biochemistry. By using the most refined methods of biochemical, physicochemical, and morphological analysis we can fill in the gaps which exist between biochemical analysis and the physiological significance of the results. Such gaps are noted in a considerable part of foreign research, especially of American and British biochemists, and even of some Soviet scientists.

The main problem in the biochemistry of the central nervous system, and especially that of brain, is to elucidate the specific characteristics of composition and metabolism which determine its biological role in maintaining the equilibrium between the living organism and its environment.

A further concern of functional biochemistry is the metabolic study of the central nervous system at various levels of activity under the influence of specific environmental conditions (nutrition, meteorological factors, etc.), taking into consideration the individual characteristics of the person or animal under investigation (age, sex, etc.).

It follows that the study of the biochemistry of the nervous system should not consider the brain in a vacuum but treat it in all its divergent states apparent under the influence of various environmental conditions, and take into account the demands of the organism entailed in its development.

Finally, functional biochemistry includes the metabolic and functional interrelations between organs and organ systems, the biochemical characterization of the central nervous system with its higher member, the cerebral cortex, and metabolic regulation. In the area of metabolic regulation chemical and nervous regulation are closely interrelated.

The concept of the functional unity of the nervous system, which also indicates the functional and metabolic unity of the organism with its environment, far from precluding, insistently demands a variety of approaches to the biochemical investigations of the different divisions of the nervous system. Just as the metabolism of the peripheral nerves differs from that of the central nervous system, so also, in the central nervous system itself, the cerebellum, the cerebral hemispheres, the white and gray matter of the cerebral hemispheres, and the different regions of the cerebral cortex are not equivalent either functionally, metabolically, or morphologically.

This follows not only from our general conceptions on the relationship between the metabolism, function, and morphology of the living organism but also from our earlier works which established the concept of the chemical topography of the cerebral cortex. The value of this term may be disputed. On the basis of our recent work one may suggest a somewhat different meaning to the term, considering the possibility of functional changes in biochemical processes in various parts of the nervous system. But in general the term reflects an objective consistency which must be accounted for in devising future biochemical experiments. Biochemists also should not neglect the data obtained by physiologists and morphologists.

The metabolic details of the nervous system and of its various elements are already present during its phylogeny and ontogeny. Therefore, in order to understand the causative links and to determine metabolic changes we should conduct comparative biochemical studies. To do this we need information on the composition and the biochemical properties of the nervous system of animals in various stages of ontogenetic development. Such information will reveal the metabolic details of the nervous system, and especially of its higher elements, in animals closely related phylogenetically but having different life patterns under natural conditions. Finally, it is important to study the biochemical changes of the nervous system during alterations in the living conditions of the organism, as well as during various physiological states (age, lactation, pregnancy).

In comparative studies of this kind methodological difficulties may lead to erroneous conclusions. There are a number of observations which are still not accounted for. At various ontogenetic stages the brain and nerve tissue contain different amounts of water. The relative weight of the brain (to that of the body) changes, and so does the weight of various other parts of the central nervous system. The weight ratio of the gray and white matter of the cerebral hemispheres may also change. All this must be accounted for before arriving at any conclusions.

Work on the physiology and the biochemistry of the brain should be conducted by both biochemists and physiologists in collaboration with morphologists and physicians.

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*BIOCHEMISTRY OF THE BRAIN**

One of the most interesting, exciting and complex areas in the field of biochemistry is that of brain biochemistry. There are many difficulties inherent in the study of the chemical composition of the brain and of the specificities of its metabolism due to the diversity of cellular and conductive structures, the complex distribution of the gray and white matter and the high percentage of labile compounds in brain tissue. There is, however, a wealth of data on the descriptive, dynamic, and functional biochemistry of the brain which enables us, to a certain extent, to gain some insight into the specificity of nervous activity.

In the present lecture I would like to present the results of our studies on brain biochemistry which were conducted for a number of years at the Institute of Biochemistry of the Academy of Sciences of the Ukrainian SSR. I would also like to present the results of several important investigations of other scientists in this area.

The most important innovation in modern physiology is the conception of a unified and plastic organism in close interaction with a changing environment, which influences its composition, development, and functional properties and forms, both in ontogeny and in phylogeny.

In higher animals, and especially in man, the unity of the organism and its interaction with the external and internal environment is regulated and organized, as shown by Sechenov and Pavlov, by the central nervous system, which coordinates all the functions of the living organism.

The most important concern of human and animal biochemistry is the metabolism of the unified organism in various functional states and under various external environmental conditions. Such metabolic investigations should elucidate the relationship between organic and systemic function and metabolic specificity in the nervous system. They should also elucidate the mechanism of metabolic regulation by the nervous system.

In our studies on brain biochemistry we tried to ascertain whether the functionally different brain components differ in chemical composition, and whether there is any change in their metabolic processes with changes in the functional state, or under the influence of various factors.

METHODS

To investigate the relationship in the brain between functional changes and metabolic processes both in vivo and in vitro studies have been

* Lecture read at the Fourth International Biochemical Congress in Brussels in 1955 (supplemented and revised) (Proceedings of the Fourth International Congress of Biochemistry, Brussels. 1955. Academic Press Inc., published New York, 1956, p.375 to p.400).

resorted to. Experiments with tissue slices or brain homogenates are of limited value in functional biochemistry. Experiments in vitro may reveal only the mechanisms of metabolic reactions whereas experiments in vivo reveal their physiological significance and their functional role.

Since brain tissue is characterized by very rapid metabolic reactions and by a high sensitivity to physiological factors, the process of preparing brain tissue slices may lead to the decomposition of a number of important compounds, such as ATP, and DPN. Moreover, the stimulation of isolated nerve elements without any natural link with other brain components may present a distorted picture.

Thus, the metabolic processes taking place in an intact brain may differ from those taking place in brain slices. This has been confirmed by the results of several experiments which show that the same factor may affect differently the biochemical reactions studied in vitro and in vivo. For example, the administration of certain barbiturates leads to an increase in the brain phosphocreatine content in experiments in vivo /1 and 2/, whereas in experiments in vitro the phosphocreatine content decreases under the influence of the same barbiturates /3/. The same is true for inorganic phosphorus. It is true that in some cases the results obtained in experiments in vivo and in vitro are similar. For example, narcotics inhibit respiration of brain tissue in experiments both in vivo and in vitro /4, 5/. Therefore, in vitro experiments may be used to obtain certain preliminary data in functional biochemistry. However, although experiments on whole animals involve many methodological difficulties, only such experiments may provide an acceptable answer to the problem of the relationship between functional changes and metabolic processes in the brain.

For studies of the chemical composition and metabolism of the brain during a given functional state, and especially of the metabolism of labile phosphorus compounds, the methods of sacrifice and fixation of the animal are of great importance.

Kerr /6/ in 1935, employed the method of freezing the brain in liquid air. He anesthetized the animal, trepanated it under artificial respiration, (small animals were not trepanated), poured liquid air on the hemispheres, and then extracted the brain in the frozen state. In using this method one has to remember that anesthesia may also produce changes in brain metabolism. In order to avoid this the animal may be rapidly decapitated, its cerebral hemispheres extracted and plunged into liquid air. Alternatively, the severed head may be plunged into liquid air and the frozen brain may then be extracted. Decapitation causes an acute stimulation of the brain accompanied by the decomposition of labile compounds. To avoid this, prior to decapitation one may administer hexenal (1 mg of a 10% solution), which produces instantaneous death. The dependence of the results of ATP and creatine phosphate determinations on the method of preparation of the brain (freezing in situ or after decapitation, anesthesia, etc.) is clearly seen from the work of Ferdman and Dvornikova /7/.

It is also possible /8, 9/ to plunge whole animals, such as rats and mice, into liquid air. However, this procedure may cause a strong, though short-lived, stimulation of skin receptors and the stimuli may reach the brain before it has been frozen, thereby producing changes in brain metabolism.

In the last two decades radioactive isotopes have been used to investigate nerve tissue metabolism. The use of radioactive isotopes established that

numerous chemical substances in adult animal brain which were thought to be stable are continuously being degraded and resynthesized.

For studies on brain metabolism the most widely used radioactive isotope is P^{32}. The reason for this is that phosphorus compounds play an extremely important part in energy-yielding reactions of nerve tissue metabolism and that phosphorylated compounds are extremely active in various transformations.

PROTEINS

That proteins play an important role in the function of the central nervous system had already been established by Danilevskii in 1891 /10/. However, not much more is known about proteins today than was known in the days of Ewald /11/, Halliburton /12/, and Danilevskii. The difficulties encountered in studies on brain proteins are due to the fact that proteins form complexes with lipids. Attempts to separate lipids from proteins by extraction with organic solvents led to the denaturation of brain proteins.

Several investigators /13/ have shown that the cerebral cortex is the richest in protein content, followed by the white matter of the brain and by the spinal cord. The lowest protein content is found in the peripheral nerves. We have studied various sections of the gray matter of the central nervous system /14/ and shown that the section which is phylogenetically the youngest and functionally the most complex — the gray matter of the cerebral hemispheres — has the highest protein content. Protein content is lower in the gray matter of the cortex of cerebellum and of the subcortical ganglia, and is lowest in the gray matter of the spinal cord.

The studies of Palladin /15/ show that the gray and white matter of the brain differ from each other not only in total protein content but also in the content of various protein fractions. There are more water-soluble proteins and fewer insoluble protein residue in the gray matter than in the white.

Recently, the use of radioactive isotopes has been employed in protein metabolism research. Friedberg, Tarver, and Greenberg /16/ studied the incorporation of S^{35} methionine into proteins of various organs in rats. They found that when S^{35} methionine was injected intravenously, its rate of incorporation into brain proteins was slower than its rate of incorporation into proteins of other organs. When methionine was injected intracisternally, (to bypass the barrier between brain and blood) the incorporation of methionine into brain proteins was much more rapid than into other organ and tissue proteins. They also found that protein turnover is best measured not by the incorporation of radioactive amino acids into proteins but by the release of radioactivity from labeled proteins.

Gaitonde and Richter /17/ assumed that the rate of protein synthesis can be evaluated by the ratio of the specific activity of protein sulfur to that of acid-soluble sulfur. They concluded that the rate of protein synthesis in the brain is relatively high (higher than in the liver).

Palladin and Vertaimer /18/, trying to determine the rate of protein turnover in various regions of the central nervous system, studied the rate of incorporation of radioactive methionine (S^{35}) into proteins of various

sections of cat brains. They found that the gray matter of the cerebral hemispheres and of the cerebellum (functionally the most complex and phylogenetically the youngest members of the central nervous system) showed the highest rate of protein turnover. The lowest rate of protein turnover was found in the spinal cord, which is functionally the least complex and phylogenetically the oldest member of the central nervous system. The rate of protein turnover in the white matter of the hemispheres was similar to that found in the spinal cord. The rate in other regions of the central nervous system was found to be intermediate between these two extreme values (Figure 8).

These results were confirmed by Cohn, Gaitonde, and Richter /19/ who studied the incorporation of S^{35} methionine into brain protein by means of autoradiography. They administered methionine intraperitoneally or intracisternally and found that the incorporation of radioactive methionine into gray matter protein is more rapid than into white matter protein.

Although the absolute value of the rate of protein turnover in the brain is unknown, the relative rate in the various brain sections has been determined to some extent.

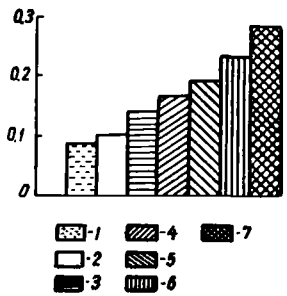

FIGURE 8. Relative specific activity of protein sulfur in various sections of the brain

1 – spinal cord; 2 – white matter of the hemispheres; medulla oblongata; 4 – midbrain; 5 – optical tubera; 6 – gray matter of the cerebral hemispheres; 7 – cerebellum

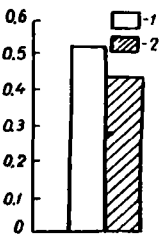

FIGURE 9. Relative specific activity of brain proteins in guinea pigs with vitamin C deficiency

1 – control; 2 – vitamin C deficiency

We have also studied the rate of protein turnover in the brain in two vitamin deficiency conditions, vitamin C and vitamin E deficiencies, with the aid of radioactive methione /18/. In vitamin C deficient guinea pigs the rate of protein turnover was somewhat lower than normal (Figure 9). However, the effect of vitamin E deficiency on protein turnover was much more pronounced. In vitamin E deficient rabbits the rate of protein. turnover in the cerebral hemispheres, the cerebellum, and the spinal cord decreased by 50% on the average. Under starvation the decrease in the turnover rate in these regions was considerably lower (Figure 10).

Nucleoproteins (ribonucleoproteins and deoxyribonucleoproteins) play an important role in the brain. Hyden obtained interesting data on nucleoproteins /20/ while studying frozen nerve cell slices by means of microradiography. While determining the content of lipids, nucleoproteins, and proteins, he found that the composition of nerve cells changed with their age; their content of nucleoproteins decreased while as that of lipoproteins increased.

During enhanced neuron activity the content of nucleoproteins decreases while that of proteins remains unchanged. Thus there are two types of alterations in the composition of nerve cells: the age-dependent changes which develop slowly, and other changes which proceed continuously and are manifest in the decomposition and resynthesis of nucleoproteins. These can be considered as the chemical basis of neuron function.

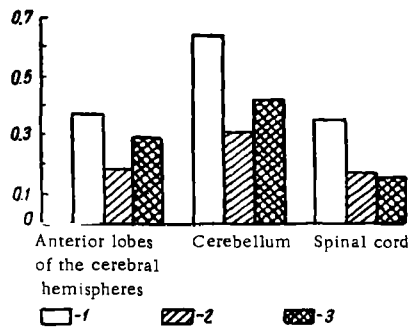

FIGURE 10. Relative specific activity of proteins from various sections of the central nervous system of rabbits in vitamin E deficiency and starvation

1 – control; 2 – vitamin E deficiency; 3 – starvation

Bulankin et al. /21/, studied age-dependent changes in the structural proteins of white rat brain and found that the content of nucleic acids decreases with age, being replaced by lipids. Similar age-dependent changes were found in whole brain tissue. At the same time the nucleoproteins became richer in protein content.

Data from the literature show that there is a correlation between the rate of protein synthesis and nucleic acid content in tissues. The high content of nucleic acids in nerve tissue leads to the assumption that they play an important role in the activity of the nervous system. This warrants the study of nucleic acid metabolism and its relation to the physiological activity of the brain.

Skvirskaya and Silich /22/ determined the nucleic acid content of various sections of the central nervous system. They found that the cerebral cortex and the white matter contained similar amounts of nucleic acid. The cerebellum contained considerably more nucleic acid, especially deoxyribonucleic acid, than the gray and white matter of the cerebral hemispheres (Figures 11 and 12).

Palladin, Rashba and Shtutman /23/ developed a method for the isolation of nuclei from brain gray matter. With the aid of this method they showed that nuclear nucleoproteins consist mainly of deoxyribonucleoprotein. It was found that the nuclei of the cortex contain 20 to 30% ribonucleic acid and 70 to 80% deoxyribonucleic acid /22/ and that the nuclei of the cerebellum contain 12 to 13% ribonucleic acid and 87 to 88% deoxyribonucleic acid. This observation led to the conclusion that there is a correlation between the composition of cellular nuclei and cell function.

Studies on the activity of the depolymerizing enzymes ribonuclease and deoxyribonuclease led to the conclusion that the metabolic rate of nucleic

acids is much higher in the gray matter than in the white. The activity of ribonuclease and deoxyribonuclease was highest in the gray matter, considerably lower in the white matter, and intermediate between these two extremes in the cerebellum.

Much attention has recently been devoted to various methods of nucleic acid determination in tissues, and particularly in the brain. It is known that fractionation of ribonucleoproteins by the method of Schmidt and Tannhauser yields nucleoproteins contaminated with various other substances /24, 25, 26/. Studies on nucleic acid phosphorus turnover in whole brain /22, 27, 28/ and in brain slices /29/ with the aid radioactive phosphorus (P^{32}) have shown that the rate of turnover of deoxyribonucleic acid phosphorus is very slow while that of ribonucleic acid phosphorus is somewhat faster, but still much slower than that of phosphoprotein phosphorus. In our studies on nucleic acid phosphorus turnover in various fractions of rabbit brain we found /22/ that the turnover rate of ribonucleic acid phosphorus is slower in the gray matter than in the white matter or in the cerebellum.

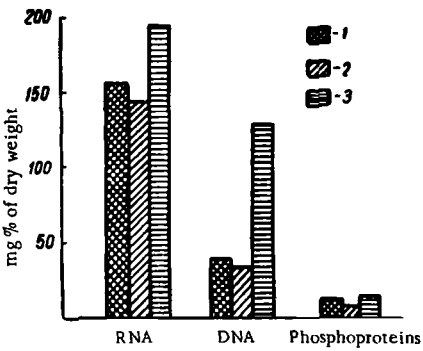

FIGURE 11. Content of some phosphorus tractions in the gray and white matters of the cerebral hemispheres and in the cerebellum

1 – gray matter; 2 – white matter; 3 – cerebellum

Similar data were obtained by Kreps /28/, who found that in rabbits the turnover rate of ribonucleic acid in the cortex is lower than in the cerebellum, midbrain, or medulla oblongata. In dogs, however, the highest rate of ribonucleic acid phosphorus turnover was found in the cerebral hemispheres. This reflects the higher functional development of the cerebral hemispheres in the dog.

TABLE 8. Activity of ribonuclease, expressed in mg P per 100 mg N hydrolyzed after one hour of incubation, and of deoxyribonuclease, expressed as the difference between the initial and final relative viscosities

Subject	Ribonuclease	Deoxyribo-nuclease
Gray matter of the cerebral hemispheres . . .	7.92	1.40
White matter of the cerebral hemispheres . .	3.03	1.00
Cerebellum	4.14	0.85

The different zones of the cerebral hemispheres of dogs, corresponding to various cortical analyzers, display different turnover rates of phosphorus compounds. The motor analyzer zone has the highest rate of ribonucleic acid and phospholipid turnover. The high turnover rate of ribonucleic acid

in the white matter of the cerebral hemispheres (and in the spinal cord) indicates that the white matter is not a metabolically inactive component of brain tissue.

The key to the understanding of the functional properties of brain tissue should be sought in protein metabolism. Of great interest, in this respect, is the phosphoprotein fraction encountered in a number of living tissues, including nerve tissue. The extremely high turnover rate of phosphoprotein phosphorus, shown in experiments with radioactive phosphorus /30, 31, 32/, indicates that phosphoproteins play an important role in the brain. The turnover rate of phosphoprotein phosphorus is much higher than that of nucleic acids on phospholipids.

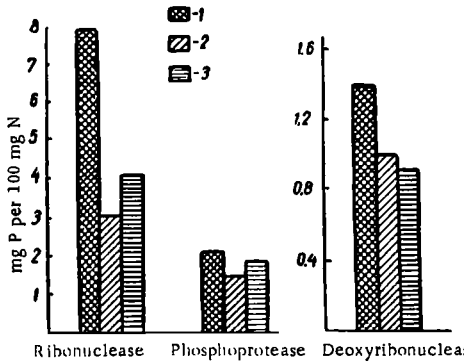

FIGURE 12. Enzymatic activity of various sections of rabbit brain

1 – gray matter; 2 – white matter; 3 – cerebellum

Engel'gardt /33/, in experiments on slices from rat brain gray matter, established that the turnover rate of gray matter phosphoproteins is much higher than that of other phosphorus compounds, including nucleic acids. He found that the phosphoprotein turnover rate depends on oxidative processes, and most of all on oxidative phosphorylation.

Brain proteins form complexes not only with nucleic acids, but also with a large number of other compounds, such as lipids and carbohydrates (glycogen).

The problem of whether lipoproteins are present in brain tissue has been adequately studied, although their presence in nerve tissue is entirely possible.

Folch /34/ isolated from the brain proteolipids soluble in organic solvents (in chloroform containing ethanol). Proteolipids differ from lipoproteins in their high lipid content. They are extremely labile compounds. Proteolipids from human and bull brain contain similar amounts of total nitrogen and phosphorus and have a similar amino acid composition /35/.

CARBOHYDRATES

Nerve tissue does not contain large reserves of carbohydrates, even though they serve as its main energy source. Brain glycogen content fluctuates within a range from 70 to 130 mg% /36/.

After the discovery of phosphorylase, which catalyzes the hydrolysis of glycogen in muscle and liver, the presence of amylase in animal tissue was denied, and former data /37, 38, 39/ confirming its presence in brain tissue were forgotten. However, the studies of Rashba /40/ of our institute have shown that brain tissue is the source of a very active amylase. This

enzyme is bound to brain proteins and is released during autolysis. The chief function of amylase is the hydrolysis of glycogen, while that of phosphorylase is the synthesis of polysaccharides.

Cori /41/ assumed that brain glycogen is formed by the combined action of phosphorylase (which synthesizes nonbranched polysaccharides of the amylose type) of another enzyme which catalyzes the conversion of amylose into the branched polysaccharide, glycogen. In our institute both these enzymes, phosphorylase and isomerase, were isolated from brain and separated by ammonium sulfate fractionation /42/. While studying the enzymes responsible for glycogen hydrolysis and synthesis we also determined the glycogen content of various brain sections /43/. He found that glycogen is present in the cortex of the cerebral hemispheres and in the cerebellum of healthy animals and that it is subject to continuous metabolic conversion.

The inaccuracy of the concept that the glycogen content of the brain is maintained at a constant level is clearly seen from results of studies on carbohydrate metabolism of the brain in various functional states; in convulsions, for example, amylase activity increases and glycogen content decreases /44/.

Prokhorova /45/ has shown that the rate of turnover of C^{14} glycogen in the brain is similar to, or even higher than that of liver glycogen. Similar results were obtained in studies on glycogen content in the cerebral cortex, the midbrain, the medulla oblongata, and the cerebellum of mice under various conditions /46/.

The question of whether glycolysis in the brain may proceed without the participation of phosphorus is of historical interest only. It is now clear that glycolysis, which is an energy-yielding reaction, may proceed only in the presence of phosphorus. Brain extracts were shown to contain intermediates of glycolysis similar to those found in muscle. Analogous enzymes of carbohydrate metabolism were also found.

We have confirmed the findings of Ochoa /47/ and others on the presence of hexokinase in the brain, both in the gray and in the white matter /48/. Hexokinase activity was found to be higher in young animals than adults /49/. We have also studied the properties and function of other enzymes present in the brain, such as phosphoglucomutase /50/, aldolase /51/, and adenosinetriphosphatase /52/. The latter has also been studied by Gore /53/. The various brain sections exhibit different rates of glycolysis. This was seen from the results of our studies on the enzymes of carbohydrates metabolism which showed that the activity of hexokinase, aldolase, phosphorylase, and adenosinetriphosphatase was highest in the cerebral cortex and in the cerebellum.

During the oxidative metabolism of glucose pyruvic acid is formed. Further oxidation of pyruvic acid in nerve tissue undoubtedly proceeds according to the tricarboxylic acid cycle.

LIPIDS

Lipids play an important role in the chemical composition of nerve tissue. They comprise almost one-half of the dry weight of the brain and

an even greater part of the spinal cord. The white matter of the brain is richer in lipids than is the gray matter. Brain lipids consist mainly of cholesterol, glycerophosphatides, sphingomyelin, and cerebrosides.

The composition of glycerophosphatides has not been adequately studied. Apart from the long known glycerophosphatides, lecithin and cephaline, nerve tissue also contains serinecephaline, obtained by Folch /54/ and acetylphosphatides, obtained by Feulgen /55/.

Acetylphosphatides were isolated from the brain by Thannhauser and his collaborators /56/. Most of the acetylphosphatides found in the brain contain cholamine.

Folch /57/ isolated from the brain a carbohydrate rich lipid called strandine which, according to Klenk /58/ is a ganglioside.

Polyakova /59/ of our Institute, determined the composition of the nonsaponifiable fraction in the gray and white matter of the cerebral hemispheres of animal and human brain by means of adsorption chromatography. She showed that in human brain sterols comprise 93% of the nonsaponifiable fraction from the white matter and 85% from the gray matter. Cholesterol is the main component in these sterols. In addition, the gray matter contains 7-oxycholesterol, not found in white matter. Thus, the functionally different brain sections differ from each other both in the quantity and in the composition of their sterols.

Experiments in vitro with brain slices have shown that rat brain is able to synthesize phospholipids /60/. Synthesis of cholesterol takes place only in brain slices from newborn rats and is totally absent in adult rat brain slices /61/. Cholesterol synthesis was also observed in experiments in vivo and was shown to decrease with the degree of growth of the rat /62/.

Recently a number of publications have appeared dealing with the isolation of various lipids from different brain sections and with the rate of incorporation of radioactive phosphorus into various lipids /63/.

The central nervous system is not a homogeneous entity. Apart from the many types of neurons with different metabolic processes, it also contains a large amount of several types of glia and various other elements. Because of this, the study of enzyme systems and of metabolic processes must be conducted with individual cells and with subcellular fractions. In this respect the combined biochemical and morphological studies of Flexner /64/, Bodian /65/ and Pope /66/ are of great value. This is also true of the recent investigations of Aboad et al. /67/ who separated the gray matter of the brain and the white matter of the spinal cord into four fractions — nuclei, mitochondria, supernatant, and lipids — and analyzed these fractions for their content of different substances (nucleic acids and a number of phosphorylated compounds) and for their enzymatic activity (oxidative, phosphorylative and several glycolytic enzymes).

COMPARATIVE BIOCHEMICAL STUDIES

A useful approach to the study of the biochemistry of the central nervous system is that of comparative biochemical investigations, that is, by studying chemical processes in the brain during ontogeny and phylogeny.

Kreps et al. /68, 69/ at the I. P. Pavlov Physiological Institute in Leningrad have since 1946 been engaged in biochemical studies of the brain during ontogenetic development. Kreps studied the development of activity in a number of enzymatic systems of the brain and compared the morphological development and functional maturation of the central nervous system with biochemical development. His studies on brain carboanhydrase in vertebrates of various ontogenetic development have shown that in the lower vertebrates the brain stem is richest in carboanhydrase whereas in the higher vertebrates the highest enzymatic activity is found in the cerebral cortex /70, 71/.

The development of brain carboanhydrase during ontogeny is characteristic for each animal species studied, reflecting the specificity of embryogenesis, and corresponds to the functional development of the nervous system /72/. One may assume that carboanhydrase has a definite physiological significance in the brain, probably connected with acid-alkali equilibrium.

In the evolution of vertebrates, proceeding from lower to higher forms of animals, there is a gradual increase in respiratory activity and decrease in anaerobic glycolysis /73/. At the same time there is an increase in the activity of cytochrome oxidase and of the entire cytochrome system in brain tissue /74/.

In mammals and birds the activity of enzyme systems participating in oxidation and phosphorylation is maximum in the cerebral cortex and considerably lower in the brain stem and spinal cord. The enzymatic activity of the cerebellum is also very high, at times equal to that of the cortex /75, 76/. These results agree with our previously obtained data which showed that the activity of enzymes involved in carbohydrate metabolism was maximal in the cerebral cortex.

Bayliss /77/ studied the activity of cholinesterase in the brain and spinal cord of young rats in the process of development.

Kreps /28/ has shown that the turnover rate of phosphoproteins, ribonucleic acids, and phospholipids differs in various regions of the central nervous system and that there is a correlation between the level of functional development and the rate of phosphorus metabolism in various brain sections.

Bieth /78, 79/ conducted comparative studies on the brain composition of adult animals from several classes of vertebrates and also investigated acid-soluble phosphorus compounds in developing rat brain (three-days to one-year-old).

Skvirskaya and Silich /22/ studied the metabolism of nucleic acids and phosphoproteins in rabbit brain during various periods of embryonic and postembryonic development. They found that the content of both nucleic acids is very high during the early stages of embryonic development and gradually decreases with the growth of the embryo. After birth the rate of decrease falls until, at one month, it levels off and the nucleic acid content approaches the normal adult level.

The phosphoprotein content of the brain is also high during the early stages of development. It decreases somewhat by the ninth day after birth, and reaches the adult level by the end of the first month (see Table 9).

Changes in the activity of brain deoxyribonuclease are concomitant with the development and appearance of new functions. Thus, for example,

the activity of deoxyribonuclease increases towards the 20th day of embryonic development, which probably relates to an increased differentiation of organs. A second rise in activity can be observed on the 9th day after birth, at the time of the appearance of vision.

TABLE 9. Nucleic acid and phosphoprotein content in the brain of rabbit embryos and of rabbits of various ages (in mg% of dry weight)

Age	Phosphorus		
	RNA	DNA	Phosphoprotein
Embryos:			
16-20 days	416	368	20.8
26-29 days	321	140	31.0
Newborn	298	148	21.7
Young rabbits:			
9-10 days	236	89	18.0
One month	195	48	3.7
Adult rabbits	166	37	6.7

METABOLISM AND FUNCTION

The brain is characterized by a very active metabolism. In the waking state the temperature of the brain is higher than that of arterial blood by 0.5°. The brain of the human adult uses about 25% of the total oxygen consumed /80/.

Neither the metabolic nor the functional activity of the brain are ever arrested. The metabolic activity of the brain may be higher or lower, depending on its degree of functional activity. Thus, it is constantly changing. The greatest changes in brain activity and metabolism can be found under experimental conditions of excitation or inhibition. Because of this the effect of these two functional states on brain metabolism has been widely studied.

Thus, for example, studies have shown that during sleep or anesthesia there is an accumulation of energy-rich phosphorus compounds, whereas during heightened activity these compounds are hydrolized, as manifest in the increased rate of glycolysis and formation of lactic acid. Olsen and Klein /81/ have shown that during convulsions produced by stimulation with an electric current the content of glucose, glycogen, ATP, and phosphocreatine in cat brain decreases, whereas the content of inorganic phosphorus and of lactic acid increases. Lactic acid content also increases during convulsions produced by camphor or strychnine /82/. It is of interest that ATP content changes less in rats than in cats.

During stimulation there is an extremely rapid degradation of phosphocreatine. The ATP content decreases only slightly since ATP is being resynthesized at the expense of phosphocreatine. The phosphocreatine content gradually returns to a normal level, 11 to 30 seconds after electric stimulation /83/.

Experiments on the action of narcotics have shown that they produce an increase in the content of ATP and phosphocreatine and a decrease in lactic acid content. Thus the changes observed during narcosis are opposed to those observed during convulsions. Narcotics also inhibit the deamination of adenylic acid /84/.

Dawson and Richter, in experiments with P^{32}, have found that the synthesis of nucleoproteins and phospholipids in mouse brain is inhibited under nembutal narcosis /85/. During sleep, the content of lactic acid in rat brain also decreases, though somewhat less than during narcosis /86/.

McIlwain et al. used the technique of stimulating brain slices with an electric current in order to study in vitro the metabolic processes of the brain during excitation /87/. Ayres and McIlwain developed a special apparatus for this purpose /88/. Their studies have shown that when brain slices are stimulated there is an increase in their respiratory activity with a concomitant increase in the acid. Also, the content of creatine phosphate decreases /89/ while that of inorganic phosphate increases /90/. Analogous results can be obtained with 2, 4-dinitrophenol. These results correspond to those obtained with experiments in vivo.

McIlwain used brain slices to study brain metabolism under conditions causing severe hypoglycemia, during which brain activity decreases. The exhausted brain tissue did not respond to electric stimuli by an increased rate of respiration when glucose was added to the medium. However, it did not lose the capacity to accumulate lactic acid from glucose /91/. McIlwain also studied the effect of various concentrations of glucose and the effect of electric impulses on respiration and glycolysis in brain slices of man, rats, and guinea pigs. He found that electric impulses increased the rate of respiration /92/.

Heald /93/ studied the conversions of creatine phosphate in cortical slices stimulated by electric impulses; he found that under these conditions creatine phosphate decomposed at the rate of 1400 m moles/g/hr after a latent period of 2-3 seconds, and that decomposition was complete after 5 seconds. The decomposition was preceded by a slight decrease in ATP content, which returned to normal by the time creatine phosphate began to decompose. When the impulses were stopped after 7 seconds, the level of creatine phosphate returned to normal after 20 seconds.

Several investigators have studied the formation of ammonia in the brain during excitation or inhibition of the nervous system. Vladimirova (1938) has shown /94, 95/ that excitation of the central nervous system with camphor is accompanied by an increase in ammonia formation and by an accumulation of lactic acid in brain tissue, while inhibition with urethane is accompanied by a decrease in the content of ammonia. Unconditioned pain stimuli (electric current), with the reflectory excitation which they elicit, also lead to an increase in ammonia content /96/. On the other hand, all inhibitory factors lead to a decrease in the ammonia content of the brain.

Richter and Dawson (1948) have also found /97/ that the ammonia content of the brain increases during convulsions caused by picrotoxin, during electric stimulation, and in anoxia, and decreases during nembutal narcosis.

In contrast to muscle tissue, adenylic acid is not the only souce of ammonia in brain tissue /98/. Ammonia may also be formed by the deamination of glutamine, with the formation of glutamic acid. During the restoration period, which follows stimulation, ammonia is removed

and the content of glutamine increases /99, 100/. Thus, the glutamine-glutamic acid system participates in the regulation of the ammonia level in brain tissue.

METABOLISM DURING EXCITATION OF NERVOUS SYSTEM

For the past several years /101, 102/, while studying brain metabolism in various functional states (some results of these studies have already been published), we have devoted particular attention to the states of inhibition and excitation of the nervous system /103, 104, 105, 106/.

Pavlov had more than once stressed that the main processes which characterize higher nervous activity are those of excitation and inhibition and that understanding them depends on the elucidation of the physical and chemical properties of the nervous system /107/.

In designing our experiments we were aware that excitation and inhibition of the brain may be produced experimentally by various means and that the metabolic processes of the brain may differ, depending upon the agent used. However, our purpose was, at first, to obtain a general idea of the main characteristics of brain metabolism during excitation and inhibition. We therefore decided to begin with the use of pharmacological drugs as agents of excitation and inhibition. Having established the main metabolic trends, we would then also resort to the use of physiological stimuli, and to unconditioned and conditioned reflexes.

In the early stages of our research on brain biochemistry we had already made use of physiological stimuli, when Gorodisskaya /108/ studied the effect of natural stimuli on proteolysis in the optical zone of the cerebral cortex and showed that the stimulation of this zone was accompanied by an increase in protein metabolism.

We have studied the metabolism of nucleic acids, carbohydrates, ATP, phosphoproteins and phospholipids. The experiments were conducted on rabbits, dogs and rats. To avoid the decomposition of labile phosphorus compounds as a result of the stimulation produced by decapitation, the animal received an intravenous injection of one ml of a 10% solution of hexenal prior to decapitation. After decapitation the brain was frozen in liquid air. Excitation was produced by pervitin which, like benzedrine, is widely used as a stimulant of the nervous system, and by cardiazol, which is also useful for this purpose.

We first studied the effect of prolonged excitation, caused by the administration of large doses of cardiazol or by an electric current, and leading to convulsions. We found that under these conditions the content of ATP and glycogen in the brain decreased /109, 110/. Our data (Figures 13 and 14) confirmed the results of Olsen and Klein, /81/ and of other investigators /1, 111/.

In experiments more closely approaching physiological conditions, where excitation was produced by a single administration of pervitin (5-7 mg per kg of body weight) or cardiazol (50-70 mg per kg of body weight) four hours prior to decapitation, we found that these stimulants produce unlike changes in brain metabolism /109/. In pervitin-induced excitation

the content of preformed lactic acid decreased, compared to control animals or to those receiving cardiazol /112/. The content of ATP and of glycogen increased after the administration of pervitin but hardly changed when cardiazol was administered. The content of ribonucleic acid also increased somewhat /113/ (Figure 15).

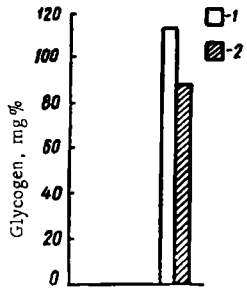

FIGURE 13. Glycogen content of dog brain during the state of convulsion

1 – control; 2 – convulsions

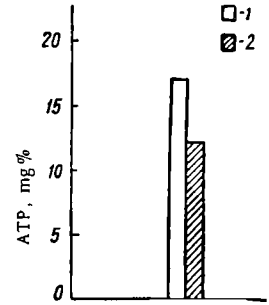

FIGURE 14. ATP content of rat brain during the state of convulsion

1 – control; 2 – convulsions

Analysis of the phospholipid content during pervitin excitation showed /114/ that there was no detectable change in the total phospholipid content and in the content of saturated and unsaturated phospholipid fractions in the interval of three hours after the administration of pervitin.

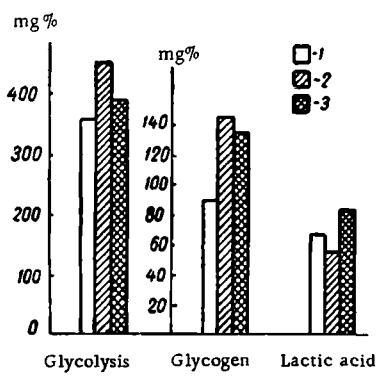

FIGURE 15. The content of glycogen and lactic acid, and glycolytic intensity in rabbit brain during excitation

1 – control; 2 – administration of pervitin; 3 – administration of cardiazol

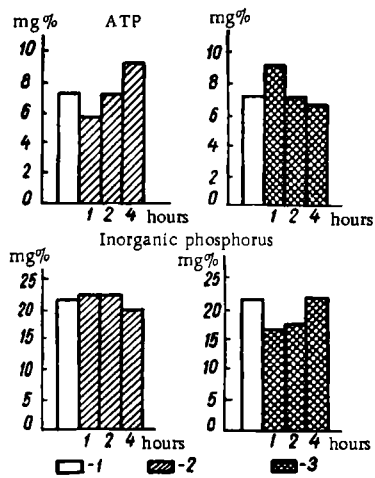

FIGURE 16. The content of ATP phosphorus and of inorganic phosphorus in rabbit brain during excitation

1 – control; 2 – pervitin; 3 – cardiazol

However, with the use of radioactive phosphorus, we were able to show that the incorporation of phosphorus into both fractions of phospholipids during pervitin excitation proceeds in a manner different from that in control animals. This shows that changes in the functional state of the nervous system also bring about changes in phospholipid turnover.

It may thus be seen from our studies that different drugs elicit different changes in brain metabolism, due to the difference in their physiological effects. In pervitin excitation the rate of carbohydrate metabolism increases, with a concomitant accumulation of ATP, which enhances the activity of the nervous system. Thus, pervitin has a stimulatory effect upon the nervous system. Cardiazol, on the other hand, brings about excitation of the cerebral cortex without increasing its activity.

Vladimirov /115/ studied the effect of excitation of the central nervous system on the rate of turnover of ribonucleic acid and phospholipids. Excitation was produced in rats by an electric current acting for a period of three hours (with intervals) on skin receptors. These studies showed that excitation of the brain resulted in an increase in the turnover rate of phospholipid and ribonucleic acid phosphorus. The rate of ribonucleic acid turnover increased by 20% and that of phospholipids by 50%.

In these experiments one must take into account the fact that the nature of the excitation may differ, depending upon the agent used and upon the duration of its action on the nervous system, We therefore determined the turnover rate of phosphorus compounds in the brain during various time intervals after the administration of pervitin or cardiazol (after 1, 2 and 4 hours).

The results of our investigations /116/ showed that the ATP content of the brain decreased one hour after the administration of pervitin, and then began to rise. After two hours it approached control values, and after four hours was considerably higher than that of control animals. These data confirmed the results of our previous experiments in which ATP content was determined four hours after administration of pervitin. In addition, they revealed the kinetics of ATP changes. Changes in inorganic phosphorus content were in an opposite direction.

Different results were obtained with cardiazol. ATP content increased one hour after administration of cardiazol and then began to fall. After two hours it was lower than in control animals, and after four hours still lower. Again inorganic phosphorus gave different results (Figure 16).

These experiments showed once again that pervitin and cardiazol, having different physiological activities, affect ATP metabolism differently.

We also studied the condition of chronic hyperexcitation, which was elicited by prolonged stimulation with electric current or by disturbance of physiological sleep. In the first instance rats were kept in a special electrode cage and were subjected daily for prolonged periods of time (1-1½ months) to an electric current of 25 to 40 V. In the second instance we kept the rats for three days in a rotating drum, rotating every 5 minutes for 30 seconds. As a result the rats were unable to sleep for three days.

These studies showed /117/ that during chronic insomnia the rate of glycolysis decreased somewhat. The content of glycogen remained almost unchanged, while that of ATP decreased (Figure 17). ATP content also decreased during prolonged stimulation with an electric current.

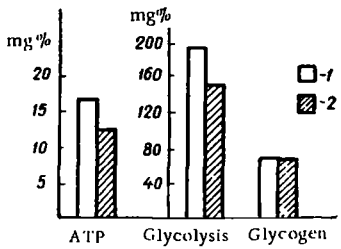

FIGURE 17. Metabolism of carbohydrates and ATP during sleep disturbance in rats

1 – control; 2 – disturbance of sleep

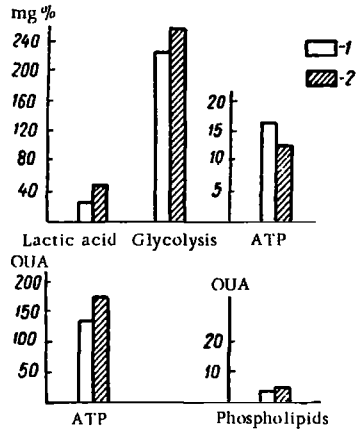

FIGURE 18. Carbohydrate and phosphorus metabolism in rat brain under normal conditions and during "disruption" of nervous activity

1 – control; 2 – "disruption" of nervous activity

During prolonged excitation with an electric current /22/ the nucleic acid and phosphoprotein content remained almost unchanged. However, by using P^{32} we were able to show that in spite of the absence of an overall change in nucleic acid content, there is a change in the turnover rate of nucleic acids during chronic excitation of the nervous system, namely, a decrease in the rate of ribonucleic acid turnover.

We have investigated a further aspect of brain metabolism during the condition of hyperexcitation — the "disruption" of nervous activity due to two opposing stimuli: conditioned alimentary (the ringing of a bell), and nonconditioned (electric current). The elaboration of conditioned reflexes in rats and the "disruption" of nervous activity were carried out according to the method of Gorsheleva /118/.

After the elaboration of the conditioned motor alimentary reflex to the ringing of a bell had been accomplished, an electric current of 20-30 volts was applied for 10 seconds simultaneously with the ringing of the bell. After 8 to 10 days of this treatment the conditioned reflex disappeared: the rats no longer ran to the source of food at the sound of the bell. Thereafter, these rats were used for the experiments.

Analyses performed on these rats showed that their content of ATP in the brain decreased and that of inorganic phosphorus increased. Lactic acid content and the rate of glycolysis also increased (Figure 18). Determination of the relative specific activity of ATP phosphorus after administration of P^{32} showed that the turnover rate of ATP phosphorus increased.

It must be assumed that in experiments of this type ("disruption" of nervous activity) we are dealing with an overburdening of the nervous system and with exhaustive stimulation.

It is thus seen that excitation of the nervous system elicited by agents which closely resemble physiological ones leads to a certain, although slight, increase in the content of ATP, inorganic phosphorus, and glycogen, and also to an increased rate of glycolysis. The turnover rate of ribonucleic acid, phosphoproteins, and phospholipids also increases, as judged by an increase in the relative specific activity of P^{32} in these compounds.

However, the action of very strong stimuli for a prolonged period of time may alter the above situation. Under these conditions exhaustion of the nervous system may take place; or, in other words, the state of excitation may pass into the state of inhibition.

METABOLISM DURING INHIBITION OF NERVOUS ACTIVITY

Inhibition of nervous activity was affected by sodium medinal or sodium amytal, two pharmacological drugs which produce narcotic sleep. The drugs were administered in small doses and the sleep produced was similar to natural sleep. The animals under narcotic sleep reacted to noise, touch, etc.

Studies on carbohydrate metabolism in rabbits during a four-hour narcotic sleep showed that the rate of glycolysis remained quite high and differed only slightly from that of control animals. Glycogen content did not increase. These data indicate that the rate of carbohydrate metabolism remains sufficiently high but that the expenditure of carbohydrates apparently diminishes (Figure 19). The content of ATP increased /109/ (Figure 20).

No changes were observed in the content of nucleic acids in rat brain during narcotic sleep. On the other hand, deoxyribonuclease activity increased. The more prolonged the narcotic sleep the higher was the activity of deoxyribonuclease.

We also studied /102/ the rate of incorporation of P^{32} into ribonucleic acids, phosphoproteins, and phospholipids of rat brain during a 24-hour narcotic sleep produced by urethane and medinal. The results showed that the relative specific activity of RNA phosphorus decreased by 27.6%, that of phosphoproteins by 19.2%, and that of phospholipids by 22.8%. Thus, it is seen that during narcotic sleep the turnover rate of RNA, phosphoproteins, and phospholipids decreases (Figure 21).

Similar results were obtained by Vladimirov /115/ who studied the effect of narcotic sleep, induced by hexanastab or amytal, on the turnover rate of phospholipid and ribonucleic acid phosphorus in rat brain. He found that during narcotic sleep the turnover rate of brain ribonucleic acids and phospholipids decreases. In the majority of cases amytal sleep was deeper and changes in the rate of RNA and phospholipid turnover more strongly pronounced.

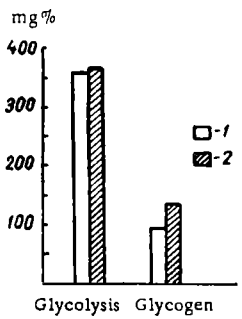

FIGURE 19. Carbohydrate metabolism in rabbit brain during narcotic sleep of four-hour duration

1 – control; 2 – narcotic sleep

The functional state of the brain had no marked effect on the content of brain phospholipids.

Thus, the state of inhibition produced by sleeping pills is characterized by a lower rate of brain metabolism: the content of ammonia decreases; the rate of oxidation, glycolysis, and glycogen turnover decreases, and so does the turnover rate of phospholipid and ribonucleic acid phosphorus. The content of ATP increases, a fact indicative of the preparation for future brain activity.

According to Pavlov inhibition is a process protecting nerve cells from exhaustion and facilitating the restoration of their function. Our data show that during narcotic sleep metabolic activity, though lower, is still quite high and that favorable conditions are created for synthetic processes, which are prerequisite for the restoration of brain activity.

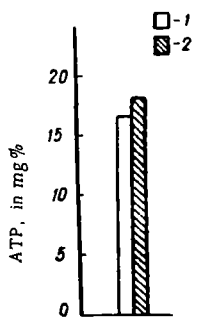

FIGURE 20. ATP content of rat brain during narcotic sleep of four-hour duration

1 – control; 2 – sleep

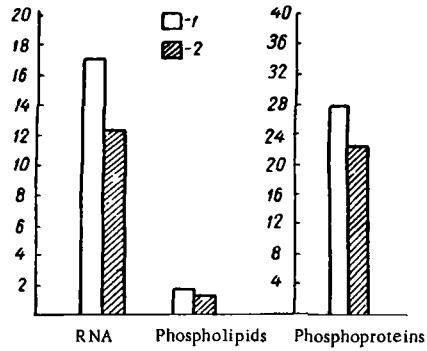

FIGURE 21. Effect of a 24-hour narcotic sleep on the relative specific activity of phosphorus compounds in rat brain. P^{32} was administered two hours before the determinations

1 – control; 2 – sleep

The above changes were observed during sleep of short duration. We asked ourselves whether these changes would remain unaltered during a prolonged narcotic sleep (e.g., of 96-hour duration). In this purpose we decided to study the metabolism of rat brain during narcotic sleep caused by subcutaneous injections of a mixture of urethane and medinal (administered twice daily during sleep of various duration — 4, 24, 48, and 96 hours).

Our experiments showed that changes in brain metabolism during prolonged sleep differed from those observed during sleep of short duration. After 96 hours of sleep the content of lactic acid increased (rather than decreased), while that of ATP did not. The rate of ATP turnover increased, as judged by the relative specific activity of ATP phosphorus (Figure 22). It may be assumed that the changes in brain metabolism during prolonged narcotic sleep are due to the toxic effect of the drugs. This assumption is strengthened by the general appearance of animals after 96 hours of sleep.

It can be concluded that during inhibition of nervous activity (narcotic sleep) degradation processes slow down, the rate of turnover of glycogen,

ribonucleic acid, and phospholipids slows down, and the content of ammonia decreases. The content of glycogen and of ATP increases. Conditions which favor synthetic processes are created, thus facilitating the restoration of brain activity after waking.

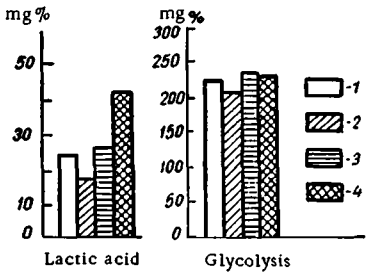

FIGURE 22. Content of preformed lactic acid, and rate of glycolysis during narcotic sleep of varying duration

1 – control; 2 – 24-hour sleep; 3 – 48-hour sleep; 4 – 96-hour sleep

I have presented here some of the main results of a number of studies, including ours, on the biochemistry of the brain, with particular emphasis on brain metabolism under various environmental and functional conditions.

From the data presented in this paper it may be seen that much has been done to elucidate the structure of functionally different sections of the central nervous system and the metabolic processes of the brain (especially carbohydrate metabolism). Some light has been shed on several problems of functional biochemistry, particularly on the biochemistry of excitation and inhibition of nervous activity, the two most important physiological states of the nervous system. Studies of this kind show that excitation and inhibition are accompanied by various, generally diametrically opposed changed in brain metabolism.

In spite of the progress in this field many problems remain unsolved. The ultimate goal of functional biochemistry is to reveal the biochemical basis for the activity of the various brain sections and to understand brain metabolism to such an extent as to be able to control it.

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*BIOCHEMICAL CHARACTERIZATION OF FUNCTIONALLY DIFFERENT SEGMENTS OF THE NERVOUS SYSTEM**

In order to understand the problems involved in the functional biochemistry of the nervous system and to elucidate the relation between specific functions of the various brain sections and their chemical composition and metabolism, studies should be conducted on the chemical composition and metabolism of the functional and structurally different sections of the central and peripheral nervous systems.

First data on the chemical composition of different sections of the nervous system were obtained at the end of the 19th century by A. Ya. Danilevskii /1/, D. Petrovskii, B. Slovtsov, A. Lents, and others. They found that the more complex the function of a specific section of the central nervous system the higher is the protein content of that reaction. According to Petrovskii /2/, the gray matter of the cerebral hemispheres contains 55.3% protein (per dry weight of tissue) and the white matter only 24.7%. Slovtsov, and A. Georgievskaya /3/ found the following protein composition in various sections of the nervous system: the cerebral cortex — 51%, the white matter of the brain — 33%, the spinal cord — 31%, and the sciatic nerve — 29%.

We have already seen that the highest protein content is found in the gray matter of the cerebral hemispheres, followed by the white matter of the brain and the spinal cord. The peripheral nerves are lowest in protein content. Thus, the more complex the function of a given section of the nervous system the higher is its protein content.

A. Palladin and E. Rashba have studied the distribution of proteins, creatine, and water in various brain sections of animals in various stages of phylogenetic development. They found /4/ that, in all animals studied, the highest protein content is found in the cerebral cortex, followed by the cerebellum. The lowest protein content is found in the white matter of the hemispheres. The differences in protein content between the different brain sections were most pronounced in mammals, that is, in animals with a highly developed central nervous system. In birds — animals with a less differentiated nervous system — these differences were less pronounced.

To confirm the relation between the complexity of function and protein content we determined the chemical composition of the gray matter of various brain sections /5/. Among the sections studied were the cerebral cortex, the subcortical ganglia, the cerebellum, and the spinal cord. The results showed that the cerebral cortex, which is functionally the most complex and phylogenetically the youngest section, has the highest protein content. The gray matter of the cortex of the cerebellum and of the subcortical ganglia is poorer in protein content. The lowest protein content was found in the gray matter of the

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spinal cord which is functionally the least complex and phylogenetically the oldest section. Similar results were obtained by Lents /5a/ who found that the gray matter of the brain was richer in protein content than was the gray matter of the subcortical ganglia.

We also determined the chemical composition of the spinal cord ganglia in several sections of the autonomous nervous system and showed that here also the phylogenetically youngest sections are the richest in nitrogenous substances /6/.

In these studies we determined the total protein content in the various sections of the nervous system. However, one may assume that the various functionally and structurally different parts of the nervous system have different specific proteins associated with them. This prompted us (Palladin and Goryukhina) to separate the proteins from nerve tissue into four fractions (water-extractable, extractable with 4.5% potassium chloride, extractable with-0.1 N sodium hydroxide, and insoluble residue) and to study the content of these fractions in the gray and white matter of the cerebral hemispheres.

The results showed /7/ that the gray matter is richest in water-soluble proteins. Water-soluble proteins comprise about 30% of the total proteins in the gray matter, whereas in the white matter they comprise about 19%. White matter, on the other hand, contains much more insoluble protein residue — 22% in the white matter versus 5% in the gray matter.

The data of N. Polyakova from our Institute are even more convincing. She studied soluble proteins from various sections of the central and peripheral nervous systems by means of paper electrophoresis. These studies /8, 9/ showed that there are about 6 to 7 protein fractions in the gray and white matter of the cerebral hemispheres, the cerebellum and the spinal cord.

The various sections of the central nervous system differ from each other both in the number of protein fractions and in the content of each of the fractions, which consist mainly of globulins. Negligible amounts of albumin were found in the central nervous system. In contrast to the central nervous system, the peripheral nerves contain considerable amounts of albumin /10-12/. Thus, functionally and structurally different sections of the nervous system differ from each other both in protein content and composition.

Copper-containing proteins have also been found in the brain. According to Porter and Folch-Pi /13/ the cerebral hemispheres are richer in copper-containing proteins than is the white matter.

In order to ascertain whether the various brain sections differ in their rate of protein metabolism we (Palladin and Vertaimer) studied the rate of incorporation of S^{35} methionine into proteins of various sections of the central nervous system of cats. We found /14/ the following average specific activities of proteins: the gray matter of the cerebral hemispheres — 0.23, the white matter of the cerebral hemispheres — 0.10, the cerebellum — 0.23, the optical tubera — 0.19, the midbrain — 0.17, the medulla oblongata — 0.14, and the spinal cord — 0.08.

Thus, the highest rate of protein metabolism is found in the cerebral cortex and in the cerebellum, which are functionally the most complex sections of the central nervous system. The lowest rate of protein metabolism is found in the spinal cord, which is functionally the least

complex section. The rate of protein metabolism in the white matter of cerebral hemispheres approaches that of the spinal cord. In the midbrain, the optical tubera, and the medulla oblongata, the rate is intermediate between these two extremes.

Similar data were obtained by Cohn, Gaitonde and Richter /15/ with rat brain. They found that the rate of incorporation of S^{35} methionine was higher in the gray matter of the brain than in the white matter.

Gaitonde and Richter /16/ also studied the rate of incorporation of radioactive methionine into brain proteins by means of autoradiography. They showed that the rate of incorporation of radioactive methionine is high in those brain sections which contain nerve cells, and low in the white matter. According to Waelsch /17/ the highest rate of protein turnover is found in the corpus callosum.

T. Silich /18/ of our Institute compared the rate of protein metabolism in nerves with that in the brain by studying the rate of incorporation of radioactive methionine into nerve proteins. She showed that the rate of protein turnover in nerves is much lower than in the brain; about one-fourth as high as that of the white matter and one-sixth as high as that of the gray matter of the brain.

Kravchins'kii and Silich of our laboratory /19/ studied the rate of incorporation of radioactive methionine into protein fractions of the gray and white matters of cat brain by the method of Mirsky and Pollister. They showed that radioactive methionine is incorporated into the various protein fractions at different rates. The highest rate of incorporation was found in salt extractable protein fractions (0.14 M and 1 M sodium chloride). The rate of incorporation into sodium-hydroxide-extractable fractions and into the insoluble residue was much lower. The rate of protein turnover of all protein fractions in the gray matter was higher than that of the corresponding protein fractions in the white matter.

Silich /18/ studied the rate of incorporation of radioactive methionine into analogous protein fractions of nerves and showed that the highest rate of incorporation was found in sodium-chloride-extractable protein fractions. The rate of incorporation into proteins extractable by sodium hydroxide was considerably lower and that into the insoluble residue lower still.

Comparative studies on protein turnover rates in various fractions of nerves and the white and gray matter of the brain showed that the turnover of all protein fractions in nerves proceeds at a much lower rate than that in the corresponding protein fractions of the white and gray matter of the brain /18/.

Thus, determinations of the total radioactivity in nerve and brain proteins, and of the radioactivity in individual protein fractions of these tissues showed that the rate of protein turnover in nerves is much lower than in the brain, and especially in the gray matter of the brain.

Much attention is devoted presently to the chemical composition and metabolism of individual cellular structures. However, the available data on biochemical properties of cytoplasmic structures are mainly for mammalian liver. Few studies have been made on subcellular fractions of other tissues, including nerve tissue. Investigations of the subcellular fractions of the nervous system are of great interest.

O. Kirsenko of our Institute studied the protein content of various subcellular fractions of rabbit brain. The subcellular fractions were

obtained by differential centrifugation. He found that the mitochondrial fraction is richest in protein (23% of the total protein content of brain tissue), followed by the supernatant fraction. The nuclear fraction contains about 7.5% of the total protein and the microsomes about 1.7%.

Aboad /20/ obtained three subcellular fractions from the gray and white matter of the brain and spinal cord: a nuclear, a mitochondrial, and a supernatent fraction. The mitochondrial fraction from the gray matter was richer in nitrogen than that from the white matter.

We also studied protein metabolism in various subcellular fractions of the nervous system /21/. We determined the rate of incorporation of S^{35} methionine into proteins of nuclei, mitochondria, microsome, and supernatant. The subcellular fractions were obtained from the cerebral hemispheres and cerebellum of rabbits by differential centrifugation. These studies showed that there are different rates of incorporation into the various subcellular fractions. The highest metabolic rate was found in the microsomal proteins. The specific activity of microsomal proteins from the cerebellum was on the average 285.4, that of microsomal proteins from the hemispheres — 200.8. The corresponding values for mitochondrial proteins were 174.1 and 97.0, and for nuclear proteins, 189.4 and 174.2. The rate of incorporation of radioactive methionine into proteins of the supernatant fraction was close to that of the microsomal proteins. It can be seen that the rate of turnover of proteins from subcellular fractions of the cerebellum is higher than that of proteins from analogous subcellular fractions of the cerebral hemispheres.

Clouet and Richter /22/ studied the rate of incorporation of radioactive methionine into proteins from subcellular fractions of young rat brain. They showed that the highest rate of turnover is found in the microsomal and supernatant fractions, and that the lowest rate is found in the mitochondrial fraction. In the microsomal fraction the highest rate of turnover was found in alkali-soluble proteins. That of water-soluble proteins was lower. In the mitochondrial and nuclear fractions the salt-extractable proteins showed the highest rate of turnover. The lowest turnover rate was found in insoluble proteins. This was true for all subcellular fractions.

The phosphoproteins encountered in various animal tissues, including nerve tissue, are of great interest. The turnover rate of phosphoproteins is extremely rapid, indicating that they play an important role in brain function (Davidson et al. /23/, Johnson /24/, Vladimirova /25/ and others). According to Engel'gardt /26/ the rapid turnover rate of phosphoproteins, which is much higher than the turnover rate of nucleic acids, is related to oxidative phosphorylation.

According to E. Skvirskaya of our laboratory the gray matter of the cerebral hemispheres and cerebellum contains twice as much phosphoprotein as the white matter /27/.

Kreps /28/ showed that the turnover rate of phosphoproteins differs in various sections of the central nervous system of animals and that there is a correlation between the level of functional development of various brain sections and the rate of phosphoprotein turnover. Thus, in dogs the turnover rate of phosphoproteins in the cerebral cortex is higher than that in other brain sections. Phosphoprotein phosphatase activity is higher in the cerebral cortex and the cerebellum than in the white matter /27/.

The functionally different sections of the nervous system also differ from each other in their content and metabolism of nucleic acid. These, according to recent data, participate in important living processes and are of paramount importance for the biological function of the cell.

Studies carried out in our laboratory /29/ showed that the cerebral cortex and the cerebellum have the highest nucleic acid content; the nucleic acid content of the cerebral hemispheres is much lower, while that of the peripheral nerves is lower still. Ribonucleic acid (RNA) content is highest in the cerebral hemispheres and the cerebellum, lower in the white matter, and still lower in the peripheral nerves. Deoxyribonucleic acid (DNA) content is highest in the cerebellum, about one-half as high in the gray matter, and much lower in the white matter and the peripheral nerves.

Skvirskaya and Babii /30/ from our Institute have studied the composition of nucleic acids from various sections of the nervous system of cats and cows, using the chromatographic method. They showed that the content of nitrogenous bases in RNA from the gray and white matter of the cerebral hemispheres and from the cerebellum is almost identical and clearly differs from the RNA of peripheral nerves, which contain more guanine and less adenine and uracil. Nerve RNA is characterized by a high coefficient of specificity $\frac{(G+C)}{A+U}$. This coefficient is almost identical for various brain sections and fluctuates within a range of 1.68 to 1.75 in cow brain, reaching a value of 2.25 in cow nerves.

In contrast to RNA, in DNA no differences in composition were found in the various brain sections and in the peripheral nerves. The content of nitrogenous bases in DNA from various brain sections and the peripheral nerves was identical. The same was true for the specificity coefficient, which was 0.67 to 0.69 for the gray and white matter of the hemispheres and for the cerebellum, and 0.7 for nerve tissue,

E. Skvirskaya and T. Silich /29/ studied the turnover rate of nucleic acids freed from contaminants, obtained by the conventional isolation procedures of Tannhauser. The highest rate of turnover was found in the gray matter of the cerebral hemispheres, as judged by the rate of incorporation of P^{32}. The turnover rate was lower in the cerebellum, and lowest in the white matter of the cerebral hemispheres.

Similar conclusions were drawn from results obtained with the depolymerizing enzymes, ribonuclease and deoxyribonuclease from various brain sections. The highest ribonuclease activity was found in the gray matter of the cerebral hemispheres. It was lower in the cerebellum and lowest in the white matter. Deoxyribonuclease activity was highest in the gray matter, lower in the white matter, and lowest in the cerebellum /29/.

Kreps et al. /28/ found that the turnover rate of ribonucleic acid differs in various sections of the central nervous system and that there is a correlation between the level of functional development of a section and its rate of ribonucleic acid turnover. Thus in dogs the RNA turnover rate in the cerebral cortex was higher than that in any other brain section.

Various zones of the cerebral cortex of dogs, corresponding to the various analyzers, have different rates of ribonucleic acid turnover (Kreps). The highest rate of RNA turnover was found in the motor analyzer zone.

According to Aboad /20/ one half of the total ribonucleic acid content is found in the mitochondria.

Determination of the RNA content in nuclei isolated from the cerebral hemispheres and the cerebellum showed that the former are richer in RNA content than the latter /30/.

The composition of RNA from nuclei of the cerebral hemispheres of cows differs from that of nuclei from the cerebellum /30/. The RNA from nuclei of the cerebellum contains more guanine and cytosine and less uracil, and its specificity coefficient is higher than that of RNA from nuclei of the cerebral hemispheres. The specificity coefficient of RNA from nuclei of the cerebellum was 1.43 and that from nuclei of the hemispheres, 1.09. DNA composition was identical in nuclei of the hemispheres and the cerebellum.

Judging from the rate of incorporation of radioactive phosphorus, the turnover rate of RNA from nuclei of the hemispheres is considerably higher than that of RNA from nuclei of the cerebellum /30/.

Thus, the functionally and structurally different sections of the nervous system differ from each other in nucleic acid content, turnover, and composition. The more complex the section the higher its turnover rate and the content of nucleic acids (the highest being in the cerebral cortex and in the cerebellum). Nerve tissue differs from brain tissue in its considerably lower content of RNA and DNA, and in the chemical composition of RNA, which has a higher specificity coefficient.

Roberts, Frenkel and Harman /31/ studied the content of free amino acids in various sections of the nervous system of mice and rabbits by means of paper chromatography. They found in the brain free glutamic acid, aspartic acid, γ-aminobutyric acid, taurine, cystine, serine, glycine, alanine, valine and leucine. The content of free amino acids was considerably lower in the spinal cord and lower still in the sciatic nerve, where γ-aminobutyric acid was absent.

Considerable attention has recently been devoted to glutamic acid and glutamine as the probable sources of ammonia in the brain, and γ-aminobutyric acid has also drawn much attention. According to the results of a number of studies the functional activity of the brain is closely linked with ammonia formation. The amount of ammonia in the brain may serve as indication of its functional state.

Glutamic acid also plays an important role in the metabolism of the nerve tissue. This conclusion may be drawn from the fact that there is more glutamic acid in brain tissue than in any other tissue. It is noteworthy that more than 50% of the total glutamic acid present in nerve tissue is in the form of glutamine. Brain tissue is second only to heart muscle in glutamine content.

The various, functionally different, sections of the central nervous system contain different amounts of glutamine and glutamic acid. Krebs, Eggleston, and Hems /32/ studied the content of glutamic acid, glutamine and ammonia in various tissues of different animals. They found that in sheep the cerebral cortex has the highest content of glumatic acid and glutamine, and that this content is higher in the gray matter than in the white.

A. Silakova of our Institute, who worked with rabbits, also found that the highest glutamine content is present in the gray matter of the cerebral hemispheres and in the cerebellum; in other words, in the functionally most active sections of the central nervous system. The white matter of the cerebral hemispheres contains about half as much glutamine as does gray matter.

Berl, Waelsch, /33/ and Tower /34/ have determined the content of glutamic acid, glutamine, and γ-aminobutyric acid in various sections of cat and rat brain. According to their data the cerebral cortex has the highest content of glutamic acid (11.6 μ moles/g of tissue in rats, and 9.55 μ moles/g of tissue in cats). Closely approaching these values are the glutamic acid contents of the nucleus caudatus (9.05 μ moles/g for cats), the optical tubera (11.7 μ moles/g in rats, 9.8 μ moles/ g in cats), and the cortex of the cerebellum (10.2 μ moles/g in rats, 9.0 μ moles/g in cats). The white matter of cat cerebral hemispheres contains only about 25 % of the glutamic acid found in the gray matter (2.25 μ moles/g). The highest glutamine content was found in the cerebellum (5.8 in rats, 4.25 in cats) and in the cerebral cortex (5.0 in rats, 5.35 in cats). The highest content of γ-aminobutyric acid was found in the optical tubera (3.5 in rats), followed by the olfactory ganglion (3.2), and the cortex (2.0).

Roberts /37/ obtained somewhat different data on the distribution of γ-aminobutyric acid. The highest content of γ-aminobutyric acid was found in the cerebral cortex (64 mg%), next in the optical tubera (41 mg%), and finally in the white matter (28 mg%).

In the central nervous system γ-aminobutyric acid is unique. In the cortex, glutamic and γ-aminobutyric acids were found mostly in the neurons, whereas about 50 % of glutamine was found in other structures /34/.

Lowe, Robins, and Egerman /38/ studied the activity of glutamic acid decarboxylase in the brain of monkeys and rabbits. They found that the highest activity was in sections of the gray matter. Decarboxylase activity in the white matter was negligible.

Proceeding from the upper to the lower segments of the spinal cord one can see a gradual decrease in the activity of glutamic acid decarboxylase. This is apparently due to the gradual decrease in the amount of gray matter from the upper to the lower segments.

The distribution of glutamic acid, glutamine and γ-aminobutyric acid differs in various subcellular fractions of cortical cells. These fractions were obtained by differential centrifugation.

According to Tower /34/ glutamic and γ-aminobutyric acids are present mainly in the mitochondria. In the nuclei the content of these acids is half of that in the mitochondria. However, mitochondria and nuclei contain similar amounts of glutamine. Neither glutamic acid, glutamine, nor γ-aminobutyric acids were found in microsomes.

Carbohydrates, the main source of energy of nerve tissue, play an important part in the central nervous system. Determinations of glycogen content in various sections of the nervous system /39/ have shown that the highest content is found in the cerebral cortex, followed by the cerebellum. The glycogen content of the medulla oblongata is about half that of the cortex, and that of the spinal cord somewhat lower than that. Least glycogen is found in the sciatic nerve.

The rate of brain glycogen turnover is very high, as judged by the rate of incorporation of C^{14} into glycogen. It is equal to or even higher than that of liver (Prokhorova /40/).

According to data obtained at our Institute the rate of glycolysis differs in various brain sections /41/. The highest rate was found in the gray matter of the cerebral hemispheres. Dikson /42/ studied the rate of glycolysis in the superficial layers of the cerebral cortex, consisting mainly

of dendrides, and in the deeper layers which comprise the bulk of nerve cells. He found that the superficial layers had a higher rate of glycolysis than the deeper ones. Dikson assumed that it is the rate of glycolysis in the dendrides which determines the glycolytic rate of the cerebral cortex as a whole. The low rate of glycolysis in the white matter of the brain and in nerves may be due to their high content of inert myelin substances.

The differences in glycolytic activity of various functionally different sections of the nervous system correspond to differences in the activity of glycolytic enzymes, isolated by us from various brain sections.;

Phosphorylase /39/ activity was highest in the cerebral hemispheres and in the cerebellum, followed by the medulla oblongata and, lastly, by the spinal cord.

The activity of hexokinase, one of the most important glycolytic enzymes was highest in the cerebellum and the cerebral cortext (Palladin and Polyakova /43/). It was lower in the medulla oblongata and in the white matter of the cerebral hemispheres.

According to Palladin and Polyakova /44/ the highest activity of aldolase was found in the cerebral cortex, followed by the cerebellum, medulla oblongata, white matter of the cerebral hemispheres and, finally, by the spinal cord.

According to Palladin and Shtutman /45/ the highest activity of adenosine-triphosphatase, an enzyme which catalyzes the release of energy stored in the phosphate bond of ATP, was found in the cerebral cortex and in the cerebellum. It was lower in the medulla oblongata and still lower in the white matter of the cerebral hemispheres. Thus it is seen that the functionally complex sections of the nervous system are characterized by a high activity of enzymes participating in carbohydrate and phosphorus metabolism.

Lipids play an important role in the chemical composition of nerve tissue, comprising about one half of the dry weight of the brain. The spinal cord has an even higher content of lipids. The distribution of lipids in various sections of the central nervous system differs from that of proteins. The highest lipid content is found in the peripheral nerves, followed by the spinal cord. The lowest lipid content is found in the brain, the white matter being richer in lipid than the gray (cortex).

Determinations of lipid distribution in the gray matter showed that the gray matter of the phylogenetically oldest and functionally most complex section — the spinal cord — has the highest lipid content (phosphatides and cholesterol; Palladin, Rashba, and Gel'man /5, 6/). The gray matter of the cerebral cortex had the lowest lipid content.

Korey /46/ determined the content of acetylated phospholipids in various sections of human brain. He found that the lowest content of these lipids is found in the cerebral cortex, nucleus caudatus, and the gray matter of the cerebellum. Large amounts of acetylated phospholipids were found in the centrum ovale and in the white matter of the cerebellum.

Polyakova /47/ studied the composition of nonsaponifiable fractions in the brain of rabbits and cows and in the white and gray matter of human hemispheres by means of column chromatography. She showed that the white matter of the cerebral hemispheres contains 40 % (of the dry weight) of the nonsaponifiable material while the gray matter contains only 8 %. She also found that sterols comprise about 93 % of the fractions of white

matter and about 85% of the gray matter fractions. In both cases the bulk of the sterols consisted of cholesterol. The gray matter contains α-oxycholesterol which is absent from the white matter. Thus the functionally different brain sections vary not only in the amount but in the composition of sterols.

Gorodisskaya /48/ determined the lipid content in various regions of the cerebral cortex and found that functionally different regions differed in their chemical composition. Thus, in addition to the anatomical and functional topography of the cerebral cortex, there also exists a chemical topography.

Aboad et al. /20/ found that the mitochondrial fraction contains about 50% of the total phospholipids and the nuclear fraction about 30%. All subcellular fractions of the gray matter have a lower phospholipid content than the corresponding fractions of the white matter.

According to Kreps, Smirnov, and Chetverikov /28/ the turnover rate of phospholipids differs in various sections of the central nervous systems and there is a correlation between the rate of phospholipid metabolism and the level of functional development of a given brain section. In dogs, the rate of phospholipid turnover in the cerebral cortex is higher than that in the white matter of the hemispheres and in other cerebral sections. In rabbits, the cerebral cortex, which is on a considerably lower level of functional development, does not exhibit a high rate of phospholipid metabolism.

The various zones of the cerebral cortex of dogs have different rates of phospholipid metabolism. The highest rate of turnover is in the motor analyzer zone.

We have already shown that the various glycolytic enzymes differ in their activities, depending upon the brain section in which they are found. The same is also true of other brain tissue enzymes.

E. Lakhno showed that oxidation-reduction reactions proceed at a higher rate in the gray matter of the brain than in the white matter. The rate of oxidation-reduction is lowest in the brain stem. Similar differences were found in catalase activity.

The studies of Aboad on the distribution of oxiding enzymes showed that their activity in the gray matter is higher than in the white matter by about 50%.

Ashby, Garzoli and Schuster /49/ studied the distribution of carbonic anhydrase, cholinesterase, and acetylphosphatase in the brain of dogs and cats. They found that the acetylphosphatase content of the brain increases during embryonic development and that it, as well as cholinesterase, appears before carbonic anhydrase. In man the activity of these two enzymes is higher in the cerebral cortex than in the white matter. Similar differences were not observed in the brain of dogs, cats, and rabbits. The nucleus caudatus of the human brain exhibits a very high cholinesterase activity. Carbonic anhydrase activity is higher in the white matter of the cerebral hemispheres than in the gray matter.

E. Kreps et al. /50/ studied the development of activity in a number of important enzymatic systems of the brain (cytochrome oxidase, succinic acid dehydrogenase and ATPase) and compared their appearance with the morphological development and functional maturation of the brain. He found that in adult animals the highest enzymatic activity is in the cerebral cortex (which is consistent with the complexity of structure and

function of the cortex). The biochemical maturation of the cortex and, in general, of the anterior higher brain sections takes place at a later stage than that of the caudal sections (medulla oblongata and spinal cord), which mature quite early (at the end of embryonic life or at the very beginning of postembryonic development).

Aboad found that there is an identical distribution of enzymes in subcellular fractions of the gray and white matters. About 80% of the activity of oxidative and phosphorylating enzymes was found in the mitochondria. About 15% of cytochrome oxidase activity was found in the nuclear fraction, where no malic acid hydrogenase was detected. Cytochrome oxidase was not detected in the supernatant fraction. Glycolytic enzymes were found mainly in the microsomes.

We have presented here the pertinent data available to us on the biochemistry of the functionally different sections of the nervous system.

From the aforegoing it is clear that the functionally different sections of the central nervous system are characterized by a number of biochemical differences: the more functionally complex the section, the higher its content of proteins (especially of water-soluble proteins, of phosphoproteins, nucleic acids, glutamic acid, α-aminobutyric acid, and glutamine; the more functionally complex the section, the higher the turnover rate of proteins, nucleic acids, glutamic acid and carbohydrates. The same is true for the activity of numerous enzymes, such as ribonuclease, deoxyribonuclease, glutamic acid decarboxylase, oxidative enzymes, and the enzymes of carbohydrate and phosphorus metabolism.

Even greater differences may be found between the central nervous system and the peripheral nerves, which have the lowest content of the above compounds and the lowest rate of their metabolism. The peripheral nerves also differ from the brain in the composition and structure of several chemical substances. Thus, for example, they contain albumin and are characterized by a different composition of RNA (higher specificity coefficient).

The subcellular fractions of nerve tissue also differ from each other in chemical composition and metabolic activity. Also the corresponding subcellular fractions from various brain sections are characterized by certain chemical differences.

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*THE USE OF RADIOACTIVE ISOTOPES FOR BIOCHEMICAL STUDIES OF THE NERVOUS SYSTEM**

The goal of biochemists interested in animal metabolism has been the elucidation of the various intermediate stages in biochemical processes. If chemical compounds involved in intermediary metabolism could be labeled in some manner, their fate in the organism could be easily followed. Biochemists have long dreamed of such a possibility.

The discovery of radioactive isotopes made this dream a reality. Labeled compounds were prepared. Their chemical and physical properties were identical to nonlabeled molecules, and their metabolism in the organism was undistinguishable from that of nonlabeled analogues. The use of radioactive isotopes opened a new era in biochemical investigation, and in particular in investigation of the nervous system which posed one of the most complex problems of modern biochemistry.

Studies on the chemical composition of the brain and on its metabolism present extreme difficulties because of the diversity of cellular and conducting structures, the complexity in the distribution of the gray and white matter, and the extreme richness of brain tissue in labile compounds. Especially difficult are studies on the relationship between specific brain function and metabolism. The correlation between the functional state of the brain and changes in brain metabolism is one of the main problems of brain biochemistry. The use of labeled compounds opened new possibilities for the study of the metabolism of the brain and of other sections of the nervous system under various physiological conditions in experiments in vivo.

The use of radioactive isotopes permitted us to establish the fact that some of the chemical substances in the brain which at first were thought to be inert were in reality very active and showed a high turnover rate. The use of radioactive isotopes also enabled us to study various metabolic pathways in the brain and to elucidate the effect of various functional states upon these pathways.

In this review I would like to present the most important results on the use of radioactive isotopes in investigations of the metabolism of the nervous system, and especially of the brain and the changes taking place in it during various functional states. I would like to present the results of studies carried out at the Institute of Biochemistry of the Academy of Sciences of the Ukrainian SSR and in other scientific institutes of the Soviet Union. I will also deal with some of the most important studies by foreign scientists.

* Lecture read at the Hungarian Academy of Sciences in 1961 and at the conference on the use of atomic energy of peaceful purposes held in Kiev in 1961 (Ukr. Biokhim. Zhurnal, Vol. 33, 1961, pp. 602-621).

Studies on the chemical nature of various brain sections show that functionally different sections differ in chemical composition, and particularly in protein content. Functionally most complex and phylogenetically youngest sections of the central nervous system are the richest in protein and also contain the highest amount of soluble compounds /20/.

Studies on the rate of protein metabolism and turnover in various sections of the nervous system were made possible only by the use of radioactive isotopes. Palladin and Vertaimer /23/ administered methionine labeled with radioactive sulfur to cats and determined the radioactivity of proteins in various sections of the central nervous system, namely in the gray and white matter of the cerebral hemispheres, cerebellum, midbrain medulla oblongata, optical tubera and spinal cord. Studies were made on the rate of incorporation of radioactive methionine into proteins of various brain sections, and in this way the rate of protein turnover in these sections was determined.

The highest rate of protein turnover was found in the gray matter of cerebral hemispheres and in the cerebellum, the functionally most complex and phylogenetically youngest sections of the central nervous system which, as previous studies have shown, have the highest protein content. The spinal cord, functionally the simplest and phylogenetically the oldest section of the central nervous system, showed the lowest rate of protein turnover and had the lowest protein content. The rate of protein turnover in the white matter of the cerebral hemispheres was similar to that of the spinal cord. In other sections, such as the medulla oblongata, midbrain and optical tubera, the rate of protein turnover was intermediate.

Similar results were obtained by Cohn, Gaitonde, and Richter who investigated only the gray and white matter of the cerebral hemispheres. They found that the incorporation of radioactive methionine into brain proteins of the gray matter was much more rapid than into white matter proteins. Later, they showed that radioactive methionine was incorporated more rapidly into those brain sections which are rich in nerve cells.

Kravchins'kii and Silich, continuing these investigations, studied the rate of incorporation of radioactive methionine into various protein fractions of the gray and white matter of cat brains. They separated the proteins of the gray and white matter of the hemispheres into four fractions: two extractable with sodium chloride, one with a solution of 1.0 N sodium hydroxide, and one insoluble in sodium hydroxide. They found that the highest turover rate was exhibited by the salt-extractable fractions from the gray matter. The sodium-hydroxide-extractable fraction and, to a greater extent, the alkali-insoluble fraction were characterized by a low rate of incorporation of radioactive methionine; in other words, they had a lower rate of protein turnover. A similar trend was found in brain white matter, but the rate of incorporation of radioactive methionine into various protein fractions of the white matter was much lower than into the corresponding protein fractions of the gray matter.

These data on protein metabolism did not tell us anything about the role of various subcellular brain fractions in protein turnover, a subject of great interest to which much attention is being devoted presently.

The first step in this direction was taken by Belik and Krachko who studied protein metabolism in the nuclear and cytoplasmic fractions of cat brains with the aid of radioactive methionine. Nuclei were isolated from brain tissue by the method of Palladin, Rashba, and Shtutman. They showed that the rate of protein turnover in the nuclear fraction was much higher than in the cytoplasmic fraction.

Palladin, Belik, and Krachko /22/ determined the rate of protein turnover in various structural components of cells from the cerebral hemispheres and the cerebellum. The following fractions were studied: nuclei, mitochondria, microsomes, and soluble cytoplasmic fraction. The various cellular fractions were obtained by differential centrifugation of homogenates prepared from the cerebellum and the cerebral hemispheres.

The results of these investigations showed that the proteins obtained from various subcellular fractions differed in their turnover rates, as judged by the rate of incorporation of radioactive methionine. The highest rate of protein turnover was found in the microsomal fraction of the hemispheres and the cerebellum. The rate of turnover in the soluble cytoplasmic fraction was almost identical to that in the microsomal fraction. On the other hand, mitochondrial proteins had a low turnover rate. A similar low rate of protein turnover was found in the nuclear fraction of the cerebellum. The rate of protein turnover in the subcellular fractions from the cerebellum was higher than in the corresponding subcellular fractions from the cerebral hemispheres.

Clouet and Richter measured the incorporation of radioactive methionine into proteins of various subcellular fractions obtained from young rat brain. They found that the microsomal fraction possessed the highest rate of protein turnover.

Furst, Lajtha, and Waelsh studied protein metabolism in various brain sections and subcellular fractions of monkeys with the aid of radioactive lysine. They found that the rate of protein turnover in the cerebral cortex, cerebellum, thalamus, and hypothalamus was higher than in the spinal cord. The highest rate of protein turnover was found in the corpus callosum. Of all the subcellular fractions the microsomal fraction displayed the highest rate of protein turnover.

Penn studied the degradation of serum albumin labeled with radioactive carbon (C^{14}) in various subcellular components of the brain. He found that the mitochondria showed the highest rate of albumin degradation. The data obtained by Penn agree with the results of Polyakova who investigated the distribution of protease in various subcellular fractions of the brain and found that this enzyme is localized mainly in the mitochondrial fraction.

Studies on the incorporation of radioactive methionine into brain proteins were also conducted by means of historadioautography /31/. It was found that the rate of protein turnover was considerably higher in the gray matter of the brain than in the white, and that the rate of protein turnover differed in neurons of various formations in the central nervous system. In the nerve cell proper the highest activity is associated with the cytoplasm of the neuron body and with the dendrites, and the lowest with the nucleolus and the axonal process.

Silich studied protein metabolism in nerves with the aid of radioactive methionine. He found that the rate of protein metabolism, or the rate of

protein turnover, in the nerve (sciatic nerve of cats) is much lower than in the brain, both in the white and in the gray matter.

To ascertain whether all protein fractions of the nerve have a similar low protein turnover rate, nerve proteins were separated into four fractions, as described previously, and the rate of incorporation of radioactive methionine into the fractions was determined. It was shown that radioactive amino acids vary considerably in their rates of incorporation into the various protein fractions; both low and considerably high rates of protein turnover were found. However, the turnover rate of protein fractions from the nerve was found to be lower than that of corresponding fractions from the white and gray matter of the brain. Both in the nerve and in the brain the highest rate of turnover was found in the soluble proteins (extractable with sodium chloride).

Radioactive methionine and glycine were also used in studies on the effect of serotonin on protein metabolism in various brain sections /11/. The results showed that serotonin decreases the rate of protein turnover in the medulla oblongata and the cerebellum, but primarily in the gray and white matter of the cerebral hemispheres. Thus serotonin has the strongest effect on protein turnover in the phylogenetically youngest sections of the central nervous system.

It was found that potassium ions inhibit the incorporation of radioactive glycine into proteins in tissue slices of white rat cerebral cortex /56/.

Carbohydrates serve as the main source of energy in nerve tissue, even though the brain does not possess large reserves of carbohydrates, including glycogen. Earlier investigators failed to detect glycogen in the cerebral cortex and the cerebellum, and until recently the view prevailed that glycogen is of no significance in nerve tissue.

Recent investigations, however, have shown that glycogen is present in various brain sections and that it undergoes rapid turnover. This has been conclusively demonstrated in experiments using radioactive isotopes. Prokhorova /32/, using C^{14} labeled glucose and showed that the turnover rate of brain glycogen is identical to or even higher than that of liver. Further studies have shown that various glycogen fractions (free glycogen, glycogen bound to proteins or to lipids) have different rates of turnover in the brain.

The use of radioactive phosphorus enabled us to show that functionally different sections of the nervous system have different rates of nucleic acid and phospholipid metabolism. Skvirskaya and Silich /40/ determined the rate of incorporation of radioactive phosphorus into RNA and phospholipids from the gray and white matter of the cerebral hemispheres and cerebellum of rabbits. They /42, 43/ also determined the turnover rate of RNA and of phospholipids in the gray and white matter of the cerebral hemispheres, in the cerebellum, and in the nerves of cats. Similar studies were conducted on various subcellular fractions of the gray matter of the cerebral hemispheres, obtained by the method of Mirsky and Pollister.

Phospholipids and RNA from brain gray matter have a higher turnover rate than those from the white matter. The lowest turnover rate is found in phospholipids from the peripheral nerves. This is partly due to the presence of metabolically inactive phospholipids, like sphingomyelin.

Kreps found (in dogs), that the rate of RNA and phosphoprotein turnover is highest in the cerebral hemispheres. He also showed that the cerebral cortex has the highest rate of phospholipid turnover.

Functionally different regions of the cerebral cortex differ from each other in the turnover rate of phosphorylated compounds. The rate of incorporation of radioactive phosphorus into phospholipids and RNA is higher in the motor zone than in the optical and, especially, the acoustic zone /14/.

Comparative studies have shown that the rate of incorporation of radioactive phosphorus into RNA and phospholipids of whole cerebral hemisphere tissue is lower than the rate of incorporation into these same substances from cerebral hemisphere nuclei /42, 43/.

Studies were made on the rate of incorporation of radioactive phosphorus into various nucleotides from ribonucleic acid of the cerebral hemispheres and cerebellum of cats (Skvirskaya and Babii). It was shown that the turnover rates of the various nucleotides differ; adenylic acid has the highest rate and guanylic acid the lowest.

Studies were made (with the aid of radioisotopes) on the turnover rates of high-polymer phosphorus compounds (nucleic acids, phospholipids and phosphoproteins) in the brain of animals of various classes. It was shown that the turnover rate of these compounds, and especially that of phospholipids, is lower in cold blooded animals /7/. However, there was almost no difference in the content of nucleic acids, phosphoproteins, and phospholipids in the brain of these animals.

Davison, et al., have published several papers on the metabolism of various lipids in the nervous system. Cholesterol metabolism was studied by injecting C^{14}-cholesterol into the yolk sac of chickens. Turnover rates were determined (in rabbits) for cholesterol, sphingomyelin, lecithin, cerebrosides, and cephalin after administration of radioactive serine. The fate of myelin lipids was studied in the brain and in nerves. On the basis of these experiments Davison assumed that the nervous system contains two groups of phospholipids: metabolically active phospholipids, and metabolically inactive phospholipids. The first undergo rapid turnover in the brain, with a rate similar to that in other tissues. Metabolically inactive structural phospholipids which are present in various anatomical structures, such as myelin sheath, may be detected in experiments of long duration.

Moser and Karnovsky studied the biosynthesis of glycolipids and other brain lipids in mice by the use of radioactive glucose and galactose.

Prokhorova and Taranova studied the metabolism of brain glycolipids by the use of radioactive acetate and glucose. They found that the turnover rate of brain cerebrosides and gangliosides is very low (60 to 100 times lower than that of glycogen).

Robertson used radioactive phosphorus to study the turnover rate of lipoproteins in various subcellular fractions of rat brain (in nuclei, mitochondria, microsomes, and the soluble fraction). He determined the specific radioactivity of the various compounds at various time intervals after the administration of radioactive phosphorus.

The metabolism of various substances in the living organisms and in various organs, including the brain, changes with the age of the organism. Of great interest are the age-dependent changes in the protein metabolism of the central nervous system as related to organic function. Until recently only indirect data were available on the age-dependent changes in brain metabolism which were based on the synthesis-degradation coefficient,

protein content in the various fractions, etc. Direct experimental data on the rate of biochemical processes in living organisms during various periods of life were obtained only with the use of radioactive isotopes.

The use of radioactive isotopes for the elucidation of changes in protein metabolism in tissues of animals of various ages at first gave contradictory results. This was due to the fact that the limitations of the method were not considered. It should be pointed out that in determinations of radioisotope incorporation into proteins the time factor following the administration of the isotope is of great importance. Sometimes, a low isotope content after a certain time interval may be due to a rapid release of the radioactive compound from the protein, which is indicative of a rapid rate of protein turnover. Likewise, a high isotope concentration in proteins may be due to a low rate of isotope release, which may indicate a low rate of protein turnover. In studies on radioactive amino acids it is extremely important to account for and analyze all the factors which determine the level of radioactivity in tissue proteins. This is especially important in experiments performed on whole animals.

Orekhovich, using heavy water, found that the rate of deuterium incorporation into proteins from various organs, including the brain, of newborn rats is higher than into proteins of the corresponding organs from adult animals.

Salganik, using radioactive methionine, found that proteins from adult animal organs, with the exception of the spleen, had a higher rate of turnover. The turnover rate of brain proteins was not studied. Salganik determined protein radioactivity 18 hours after the administration of radioactive methionine.

Toropova determined the rate of radioactive methionine incorporation into proteins of various organs and found that the rate of protein turnover was higher in young rats. Similar results were obtained by Greenberg in experiments in vitro with brain homogenates from rat embryos and from young and adult rats.

The incorporation of radioactive methionine into brain proteins of rats of various ages was studied by Richter. He found that young rat brain possesses the highest rate of protein turnover. Age-dependent changes in brain protein turnover were also studied by Panchenko.

Palladin, Belik, and Krachko /21/ studied brain protein metabolism in four age groups of rabbits: newborn, 11—12-day-old; one-month-old, and adult rabbits. The radioactivity of brain proteins was determined after various time intervals following the administration of C^{14} methionine (after 2, 5, 14, and 24 hours).

These studies have shown that protein metabolism differs in the four age groups of rabbits, as judged from the rates of incorporation of C^{14} methionine. It is highest in newborn rabbits and decreases with the age of the animal. The turnover rate is lowest in adult rabbits. Thus, the use of radioisotopes enabled scientists to show that the rate of brain protein turnover is age-dependent.

The question arose whether the age-dependent changes in protein turnover are also manifest in intracellular fractions of brain tissue. The elucidate this probelm Palladin and Belik studied the rate of protein turnover in nuclei, mitochondria, microsomes, and cytoplasm from the brain of rabbits of various ages (newborn, 11—12-day-old, one-month-old, and adult rabbits). Protein turnover was determined by measuing the rate of incorporation

of radioactive methionine into proteins. Subcellular fractions were obtained by differential centrifugation of brain homogenates.

These studies showed that the protein turnover rates in the majority of cellular fractions (nuclei, mitochondria, and soluble fractions) differ in the various age groups. The highest rate of protein turnover was found in newborn rabbits and the lowest in adult rabbits. In rabbits 11 days to one month old the turnover rate was intermediate. No age-dependent changes in the rate of protein turnover were found in the microsomal fraction.

Adult rabbits also showed large differences in the rate of protein turnover in various subcellular fractions, which were similar to those observed in adult cats (see above). The highest protein turnover rate was found in the microsomal fraction, and the lowest in the mitochondrial fraction. In one-month-old rabbits these changes were insignificant. A low rate of protein turnover was found in the mitochondrial fraction of newborn and 11-day-old rabbits.

The use of radioisotopes permitted us to discern the presence of age-dependent changes in the turnover rates of brain phosphorus compounds. Thus, for example, the turnover of brain nucleic acids during embryonic and postembryonic development was studied by Skvirskaya and Chepinoga. These scientists studied the incorporation of radioactive phosphorus into brain nucleic acids of rabbit embryos during enhanced differentiation (16-20 days) and during the last days of embryonic life (26-29 days), in newborn animals, and in 9—10-day-old and one-month-old rabbits. They found that during the earlier stages of embryonic development the turnover rate of ribonucleic acid is highest. The turnover rate then decreases and remains low during the first day after birth. Thereafter it increases again. Similar data were obtained by Skvirskaya and Silich /40/.

The rate of incorporation of radioactive phosphorus into RNA changes towards the ninth day after birth, parallel with the change in RNA content.

According to Smirnov and Chetverikov the incorporation of radioactive phosphorus into various phosphorus compounds of the cerebral cortex of rabbits sharply decreases during the first weeks of postembryonic development. Thereafter the rate of decrease is much less pronounced. The turnover of phospholipids in rat brains is also highest during the early stages of postembryonic development, and decreases with age /57/.

The rate of glycolipid turnover in the brain of white rats of various ages was studied with the aid of radioactive acetate and glucose. Prokhorova and Taranova found that in growing rats the turnover of cerebrosides is considerably higher than in adult animals, especially during the period of myelinization. The turnover of gangliosides begins to decrease from the age of 6 months.

Kometiani and Tkekhelashvili* studied the turnover rate of brain phosphocholine and phosphoethanolamine and found that their turnover rate is higher than that of phospholipids and ribonucleic acids, but lower than that of phosphoproteins. If it is true that the turnover rate of phosphocholine and of phosphoethanolamine is higher than that of phospholipids it must be assumed that these compounds are formed not as the result of phospholipid degradation but by a direct phosphorylation of choline and ethanolamine.

* [L. K. Tkeshelashvili, in the bibliography.]

Radioisotopes were also used for the elucidation of the characteristics of brain metabolism in various functional states, and especially during excitation and inhibition of nervous activity, the two main physiological states of the central nervous system. Studies on excitation metabolism have shown that in a number of cases there were no marked changes in the content of various brain substances and in the activity of enzymes involved in the metabolism of these substances. Only the use of radioactive isotopes permitted us to disclose the characteristics of brain metabolism during excitation. It may be assumed that since brain proteins play an important role in the function of the nervous system, their metabolism should change during various functional states of the brain, and particularly during excitation.

And indeed, the studies of Nechaeva showed that during excitation of the central nervous system of rats, produced by the electrical stimulation of skin receptors, the rate of incorporation of radioactive methionine into brain proteins increased. This increase was observed only in those cases where excitation was clearly pronounced. During the appearance of inhibition symptoms, produced by overexcitation, the rate of protein turnover was the same as resting in animals.

Different results were obtained by Gaitonde and Richter. In their experiments excitation, leading to convulsions, was produced by an electric current. Only slight changes in the rate of incorporation of radioactive methionine into brain proteins were noted; these changes manifested themselves mostly in a decreased rate of incorporation. It is possible that in these experiments overexcitation, which led to inhibition, was observed. In addition, as we have shown above, the results may depend upon the time interval after the administration of radioisotopes. This necessitates the careful control of the time factor in similar experiments, and the determination of the rate of incorporation of radioisotopes at various time intervals after their administration.

Palladin, Belik, and Krachko /21/, studying the incorporation of radioactive methionine into brain proteins during excitation produced by phenamine, determined the incorporation of S^{35}-methionine after various time intervals following its administration. An increased rate of incorporation was found only in those cases where the determinations were made $2\frac{1}{2}$ hours after the administration of radioactive methionine. When determinations were made 12 hours after the administration of methionine no change in the rate of incorporation was found. This is due to the increased rate of protein turnover during excitation, which leads to a rapid release of radioactivity.

The effect of cardiamine-induced excitation on the rate of incorporation of radioactive methionine into brain proteins was studied by Zakharov and Orlyanskaya. They found that the rate of protein turnover increased during excitation. These studies have shown that the rate of protein turnover increases during excitation.

Similar conclusions were reached by Geiger, et al., who found that the rate of incorporation of radioactive carbon into brain proteins increased during convulsions. Such an increase was observed not only in radioactivity determinations on total brain proteins, but also in studies on the radioactivity of various subcellular fractions.

Shapot found that the rate of incorporation of radioactive methionine into rat brain protein decreased as a result of exhaustion produced by phenamine-induced excitation. If, however, the rats fell asleep after excitation, the rate of incorporation of radioactive methionine into brain proteins increased considerably.

Studies were also made on rat brain protein metabolism following repeated epileptic fits of the rats. These studies were conducted by Pogodaev, et al. with the aid of radioactive methionine and tyrosine. They found that the rate of protein metabolism varied, depending upon the frequency of epileptic fits. Upon single or repeated epileptic fits, which did not produce exhaustion, the rate of protein metabolism in the cortex, subcortical regions, and the cerebellum was higher than in control animals.

According to Prokhorova and Tupikova /35/, during caffeine-induced excitation the specific activity of brain glycogen decreased, while the relative specific activity remained unchanged in some experiments and decreased in others. According to Prokhorova, the glycogen content of the brain decreased during excitation.

By the use of radioactive phosphorus it was possible to show that during excitation the turnover rate of ribonucleic acids and of phospholipids increases /3, 4/.

Skvirskaya and Silich /39/ studied the effect of pervitin-induced excitation on RNA turnover. No change in RNA turnover was found.

Experiments on dogs /46, 47/ showed that sound stimulation resulted in an increase in the rate of ribonucleic acid and phospholipid turnover in the acoustic zone of the cerebral cortex. No changes were observed in the turnover rate of these compounds in the motor zone, or in other zones of the cerebral cortex.

Cardiamine-induced excitation also affects the metabolism of brain phosphoproteins and phospholipids /9/ or results in an increase in their turnover rate. Similar data were obtained by Torda.

During prolonged excitation (chronic overexcitation) the rate of incorporation of radioactive phosphorus into brain RNA decreases /40/. A similar decrease was found during induced insomnia (in rats). One may assume that the reason for this decrease is related to the weakening of the functional activity of the nervous system during stress.

Thus, with the aid of radioisotopes we have been able to show that excitation of the brain results in an increased rate of metabolism of proteins, phosphoproteins, nucleic acids, and phospholipids.

The rate of brain metabolism varies with the strength and nature of the stimuli; excitation may pass into overexcitation, which leads to exhaustion and inhibition.

The state of inhibition may be produced by various pharmacological drugs; some of these produce a state which resembles the natural, physiologic state of inhibition occurring during sleep. Such is the action of several narcotics when administered in small doses. Other drugs produce a deeper inhibition, such as is observed during sleep narcosis or anesthesia.

The use of radioactive isotopes proved fruitful in studies on the changes in brain metabolism during inhibition. Numerous studies were made on brain protein metabolism during inhibition of nervous activity.

Fridman and Pogosova, in experiments on rabbits, studied the effect of narcotic sleep produced by a mixture of urethan and veronal and found that the rate of incorporation of radioactive methionine into brain proteins remained unchanged. Nechaeva, on the other hand, found that the rate of incorporation of radioactive methionine into brain proteins decreased during narcotic sleep produced by amytal.

Similar results were obtained in studies on the effect of inhibition on the rate of incorporation of radioactive glycine into brain proteins /5/. It has been shown that during narcosis the incorporation of glycine into brain protein of rats decreases.

Gaitonde and Richter studied the rate of incorporation of radioactive methionine into brain proteins of rats during ether narcosis (of three hours' duration) and during narcosis produced by the injection of nembutal. They showed that under these conditions the rate of incorporation of methionine into brain proteins decreased considerably. The combined activity of anesthesia and lowering of body temperature led to a considerable decrease in the rate of incorporation of radioactive methionine. Mitev also observed a decrease in protein metabolism during narcosis at low temperature.

The results of experiments on inhibition depend not only on the nature and potency of the drug producing the inhibition, but also on the time lapse between the administration of the labeled drug and the measurement of radioactivity.

Palladin, Belik, and Krachko /21/, studying the turnover rate of brain proteins during inhibition, determined radioactivity at various time intervals following the administration of methionine. In these studies narcotic sleep was produced in white rats by the subcutaneous injection of a medinal-urethan mixture. The results showed that after narcotic sleep of 20 hours' duration, the turnover rate of brain proteins remained unchanged, as compared with control animals.

Prokhorova and Tupikova, using radioactive glucose, found that during narcotic sleep produced by chloral hydrate and morphine-ether there was a considerable decrease in both the specific activity and the relative specific activity of glycogen; in other words, there was a decrease in the rate of glycogen turnover. Since glycogen content thereby increases, Prokhorova and Tupikova concluded that during narcotic sleep the rate of glycogen synthesis decreases and glycogenolysis is inhibited.

Dawson and Richter found that during nembutal narcosis the rate of incorporation of radioactive phosphorus into the nucleoproteins and phospholipids of mouse brain decreases, which indicates that narcotic sleep inhibits the synthesis of nucleoproteins and phospholipids. Narcotic sleep of 24 hours' or of 9 days' duration, produced by the administration of a urethan-medinal mixture, results in a decreased turnover rate of ribonucleic acid, phosphoproteins, and phospholipids in rat brain /42, 43/.

Similar results were obtained with rats when narcotic sleep was produced by hexanastub or amytal /3/. Amytal-induced sleep was deeper and the decrease in the turnover rate of ribonucleic acid and phospholipids more pronounced. The elucidation of the effect of inhibition on phospholipid metabolism became feasible only with the use of radioisotopes, since the phospholipid content of the brain does not change during narcotic sleep.

According to Prokhorova and Taranova the turnover rate of cerebrosides decreases during narcotic sleep produced by chloral hydrate.

The turnover rate of ribonucleic acids and of phospholipids also decreases during natural sleep. Studies on the metabolism of RNA and

phospholipids in various zones of dog cerebral cortex during natural and narcotic sleep showed that during natural sleep the turnover rate decreased considerably in the motor and optical zones, but remained almost unchanged in the acoustic zone. It may therefore be assumed that in dogs the acoustic zone of the cortex is less inhibited during natural sleep than the motor and optical zones. It should be mentioned that dogs' sleep is very sensitive and that they react during sleep to the slightest round stimuli.

Comparative studies on metabolic changes during natural and narcotic (amytal) sleep, when numerous reflexes remained and the dogs could be awakened by external stimuli, showed that normal (physiological), and artificial (narcotic) sleep have similar effects on the metabolism of phospholipids and RNA in the motor and optical zones. In the acoustic zone the metabolic rate of these compounds was lower during narcotic sleep than during natural sleep. Apparently, true narcotic sleep causes a deeper inhibition in these zones than normal sleep. Indeed, during narcotic sleep dogs do not react to weak sound stimuli. During deep narcosis, produced by large drug doses, the turnover rate of phospholipids and of RNA is lower still, indicating a deeper state of inhibition.

Changes in the functional state of the brain can also be observed in various vitamin deficiencies, each vitamin deficiency having a different effect upon brain metabolism. This is clearly seen from the results of studies on the turnover of brain proteins during vitamin C and vitamin E deficiencies /23/.

During vitamin C deficiency a slight decrease is observed in the rate of protein turnover in guinea-pig brain. Vitamin E deficiency has a greater effect on protein metabolism in rabbit brain, considerably decreasing the rate of protein turnover in the cerebral hemispheres, the cerebellum, and the spinal cord by about 50% (Figure 8) [apparently in /23/.]

During vitamin A deficiency the incorporation of radioactive methionine into brain proteins decreases. If animals with vitamin A deficiency are given vitamin A 8 hours prior to the injection of radioactive methionine, the rate of incorporation of this amino acid into brain proteins increases /8/.

Palladin, Polyakova, and Gotovtseva studied the effect of starvation on the rate of incorporation of radioactive methionine into brain proteins of rabbits. They found that when young rabbits were subjected to starvation, there was a decrease in the incorporation of radioactive methionine into both soluble and insoluble brain proteins. In experiments with adult rabbits no changes in the rate of brain protein turnover were found during starvation.

Radioisotopes were also used for studies on brain metabolism during hypothermia. Vladimirov /4/ studied the effect of hypothermia on the metabolism of brain proteins by determining the rate of incorporation of radioactive glycine, methionine, and tyrosine. He found that during hypothermia there is a sharp decrease in the rate of incorporation of these amino acids into brain proteins. Nikulin found a decrease in the rate of protein synthesis in the cerebellum during hypothermia.

Vladimirov studied the effect of hypothermia on lipid metabolism with the aid of radioactive phosphorus. He found that hypothermia inhibits the turnover of phospholipids, lipoproteins, and phosphoproteins. The inhibition of phospholipids turnover was most pronounced. The decrease in the incorporation of radioactive phosphorus into hexose phosphates was less pronounced than that into phospholipids.

Chagovets, Lakhno, et al., found that during hypothermia produced by aminazine there is a lower rate of incorporation of radioactive phosphorus and thiamine into brain creatine phosphate. The combined effect of aminazine and low temperature leads to a decrease in the metabolism of ATP and creatine phosphate in rabbit brain (Shtutman).

Volkova found that during hypothermia the turnover rate of ATP and creatine phosphate in tortoise brain decreases.

Radioactive isotopes were also used in studies on brain metabolism during hypoxia. Prokhorova, Brodskaya, and Sokolova, using C^{14} glucose, have found that during hypoxia produced by sodium nitrite the rate of glycogen metabolism decreases, as judged by the lower specific activity of glycogen.

Kreps, Smirnov, and Chetverikov studied phosphorus metabolism in various brain sections during oxygen starvation. These experiments were conducted in vitro using brain slices and homogenates /13/. The results show that during oxygen starvation the synthesis of phospholipids, nucleic acids, and phosphoproteins is arrested. The effect of hypoxia on the metabolism of these compounds in the whole organism was also studied with the aid of ultraviolet microscopy /15/, Marked changes were detected in the content and distribution of these compounds in various cellular components of the brain.

Chetverikov /54/, using radioactive inorganic phosphate, found that during severe oxygen starvation in rats, under conditions where the hypoxic factor cannot be compensated for by various protective mechanisms, there is a marked inhibition in the metabolism of brain phosphoproteins and phospholipids. The highest sensitivity towards hypoxia is found in phospholipids whose metabolic inhibition can be detected before that of nucleic acid or phosphoproteins.

The turnover rate of soluble and insoluble brain glycogen fractions during gamma irradiation was studied by Brodskaya and Prokhorova, who found that the specific activity of glycogen, and especially of desmoglycogen, decreased considerably after irradiation.

From the aforegoing it is clear that radioisotopes have played an important role in studies on the metabolism of the nervous system as a whole, and of the brain in particular. Of special significance have been studies on protein metabolism, since proteins play an extremely important part in the function of the central nervous system. It has also been possible, with the use of radioisotopes, to detect changes in brain metabolism during various functional states.

There is no doubt that further use of radioisotopes will enable us to penetrate even deeper into the biochemical processes taking place in the nervous system, so as to make their control feasible. This is the ultimate goal of brain biochemistry.

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*PROTEINS OF THE NERVOUS SYSTEM, THEIR METABOLISM AND ROLE IN NERVOUS ACTIVITY**

When the chemical composition of the brain was first studied, lipids received most attention since they were thought to be of prime importance in the activity of the nervous system.

Thudichum, who may be considered the pioneer of systematic studies on the chemical composition of the brain, published a monograph in 1884 in which he ascribed to lipids the primary role in brain tissue. He was justly criticized by Alexander Danilevskii, who was the first to stress the extremely important role of proteins in the function of the brain.

During the last 15-20 years much attention has been paid to proteins and their metabolism in the nervous system. However, even now there are insufficient data on the brain proteins, which make up about 40% of the dry weight of the brain.

Using various methods for the extraction of proteins from brain tissue, recent investigators have described various protein fractions; among them albumin, globulin, neuroglobulin, neurostromin, neuronin, and others. However, upon further study, these were shown to be impure and represent a mixture of various proteins.

Brain tissue contains nucleoproteins both ribonucleoproteins and deoxyribonucleoproteins. Further studies showed that the neuroglobulin of Danilevskii was deoxyribonucleoprotein, whereas the neurostromin was shown to be ribonucleoprotein. Nucleoproteins may be complexed with lipids, such as in the liponucleoprotein isolated by Folch and Uzman.

Brain proteins complexed with lipids form two groups of compounds: lipoproteins, which are water extractable and decompose upon denaturation, and proteolipids, described by Folch and Lees, which are soluble in organic solvents such as chloroform and methanol. Folch and Lees isolated from the white matter of the brain three proteolipids, one of which, containing 50% protein and 50% lipid, was obtained in crystalline form. It is postulated that in lipoproteins the lipid is surrounded by protein, whereas in proteolipids the protein is surrounded by lipid. Proteolipids participate in the formation of myelin sheath and are resistant to proteases.

Neurokeratin belongs to the proteins of nerve tissue which are water-insoluble and resistant to proteolytic enzymes. It is found in the sheaths of nerve fibers. It differs from conventional keratin in its amino acid composition.

Brain tissue and peripheral nerves also contain collagen and elastin which are similar to the collagen and elastin of other tissues. They are present in the walls of blood vessels. There is more collagen in the

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gray matter than in the white. According to Logan, the collagen nitrogen of the peripheral nerves comprises about 35% of the total nitrogen content.

Brain tissue also contains small amounts of such proteins as cerebrocuprein, which contains two atoms of copper per molecule.

Another group of brain proteins are the phosphoproteins, which are characterized by the presence of phosphorus in the form of phosphoserine. In alkaline media the phosphorus of phosphoproteins is easily released as inorganic phosphate.

Phosphoproteins are characterized by a very high turnover rate, significantly higher than that of phospholipids or of nucleic acids. The turnover of phosphoproteins is closely associated with oxidation, glycolysis, and oxidative phosphorylation

Studies on protein composition in various sections of the nervous system have shown that eveywhere the functionally most complex and phylogenetically youngest formations are richest in protein. The following sections were investigated: the white and gray matter of the cerebral hemispheres, the functionally and phylogenetically different segments of gray matter, and the ganglia in the spinal cord and in some sections of the autonomic nervous sytem.

The gray and white matter of the brain differ from each other not only in total protein content but also in the type of proteins they contain. The gray matter is richer in water-soluble proteins than is the white matter. The soluble proteins may be extracted with water, aqueous KCl, or aqueous NaOH.

The protein composition of the various sections of the nervous system was determined by means of zone electrophoresis, which has been successfully employed for the separation of serum proteins. Soluble proteins of nerve tissue have been studied by means of zone electrophoresis since 1954. In one of the first experiments of this type Cupps employed this method to study the pathogenesis of the swelling of brain tissue proteins.

Demling, et al., studying proteins in various tissues in order to elucidate the site of formation of blood serum proteins, have also employed the method of electrophoresis for the separation of brain proteins.

Serum proteins and other tissue liquids may be separated by means of paper electrophoresis without any preliminary processing. They can be applied directly to paper strips for electrophoretic runs. For electrophoretic studies of tissue proteins, the protein must first be extracted from the tissue. The extraction should be carried out under such conditions so as to obtain a quantitative yield of the native, undenatured protein at a relatively high protein concentration — about 2% or higher. For this purpose the above investigators used various methods of protein extraction, the first stage being the autolysis of the brain tissue. Brain tissue was also ground with sand or frozen in dry ice.

The difficulties encountered in the electrophoretic separation of tissue proteins increase if the extracted proteins are contaminated with nonprotein material which may affect the electrophoretic mobility of the proteins. Thus, for example, if the protein extract contains lipids they may be adsorbed on the paper and prevent the migration of proteins in the electric field.

We frequently encountered these difficulties in investigations of brain proteins, since the brain contains large amounts of lipids. Thus, in electrophoretic studies of brain proteins special attention should be paid

to the choice of methods and conditions for the extraction of proteins from nerve tissue.

Polyakova and Gotovtseva conducted preliminary experiments on different methods for the extraction of soluble brain proteins prior to electrophoresis. They employed a number of buffer solutions with varying hydrogen ion concentrations for this purpose. Their investigations showed that the lowest protein yield was obtained when extraction was performed with buffers of low pH (3.6-5), while the highest yield was obtained by extraction with alkaline buffer solutions of pH 8.6–9.2. However, the proteins extracted in this manner were not well resolved by paper electrophoresis.

Of all the methods studied, the best one for the extraction of brain proteins was that which utilized physiological solutions of sodium chloride (saline) followed by the freezing of the homogenate in liquid air. This method yielded about 10% of the total brain proteins, and the proteins extracted in this manner were well resolved by paper electrophoresis.

Our results agreed with those of Lees, Baron, and Folch, who studied the effect of pH and ionic strength on the extractability of brain proteins.

In preparation for electrophoretic runs the various sections of the nervous system were freed of membranes and blood vessels, well washed, and homogenized in physiological saline solution. The proteins were then extracted at 2-4°C for two hours. The homogenates were then frozen in liquid air for 15 minutes and kept in the cold overnight. After thawing the extracts were centrifuged at 8000 revolutions per minute for 20 minutes.

The extracts of soluble proteins were subjected to electrophoresis on Whatman No. 1 filter paper (or corresponding filter papers of other brands), in a veronal/medinal buffer of pH 8.6, at 260 V, for 6-7 hours. The paper strips were then dried and the proteins were stained with amido-black 10 B.

The protein content of the extracts was determined, after digestion with concentrated sulfuric acid, by means of the Winkler reagent. Serum proteins from the same animal were run as a control together with nerve tissue proteins. Equal protein concentrations from each extract were applied to the paper strips. Curves were obtained with the use of a densitometer which permitted us to calculate the percent composition of each fraction.

Using the above methods, we have studied the proteins of the gray and white matter of the cerebral hemispheres, the cerebellum, and the spinal cord of cats. We have succeeded in resolving these proteins into 6-7 fractions /21/. The great majority of these fractions were found to have electrophoretic mobilities characteristic of serum globulin. A very small number of proteins were found with electrophoretic mobilities of serum albumin.

These studies have shown that the globulins form the bulk of brain proteins. The low amount of albumins in brain tissue is also characteristic of other tissues and is one of the distinguishing traits of tissue proteins, as opposed to serum proteins (Demling and Cupps).

We (Polyakova) succeeded in obtaining 7-9 protein fractions from the soluble brain proteins by means of electrophoresis. These fractions were obtained from the gray and white matter of the cerebral hemispheres, cerebellum, medulla oblongata, spinal cord, horns of the spinal cord, and the sciatic nerve of cows. It was found that cow brain is poorer in proteins with an electrophoretic mobility of serum albumin than is the brain of cats.

Analogous data were obtained by other investigators who used paper electrophoresis for the separation of soluble brain proteins.

Electrophoretic studies on proteins from the sciatic nerve of cats and cows have shown /29, 30/ that the peripheral nerves contain both globulins and albumins. In addition, the sciatic nerve contains proteins which move towards the cathode /23/. Such proteins could not be detected in the brain or in the spinal cord.

Since the nerve was carefully separated from blood vessels and was well washed with saline solution, the albumin found cannot be regarded as a contamination by serum albumin. Since nerve contains lymphatic vessels and albumin is found in lymph, we considered the possibility that the albumin detected came from the lymphatic vessels and was not a part of the tissue.

To test this postulate mobility we carefully removed the lymph and, after freezing the nerve, removed the epineurium together with the bulk of the lymphatic vessels. The protein extracted after this procedure again showed the electrophoretic mobility of albumin.

Both literature data and our results showed that albumin is present in connective tissue. We wanted to see whether the albumin present in the nerve stem is found only in the connective tissue sheaths, or whether it is present in the nerve fibers. To do this, we isolated the nerve fibers from the surrounding connective tissue and studied the electrophoretic behavior of the soluble proteins from the nerve fibers and from the connective tissue. The connective tissue was carefully separated and removed under the microscope. The extent of purification of the nerve fibers was determined by appropriate staining.

Proteins from the intact sciatic nerve of cows, and those from the isolated nerve fibers and connective tissue of the epineurium and perineurium were subjected to electrophoresis. The results showed that both the nerve fibers and the connective tissue of the nerve contain albumin. We were thus able to show that nerve fibers contain albumin.

Deiticke separated bull nerve protein into two fractions by ultracentrifugation. One was a slowly sedimenting fraction with a high electrophoretic mobility, and the other a rapidly sedimenting fraction with a low electrophoretic mobility. He assumed that these proteins are present in the nerve fiber proper.

As we have already mentioned, the nerve also contains protein fraction which moves towards the cathode. This protein is present in the nerve fibers but not in the connective tissue.

The horns of the spinal cord of cows also contain albumin but its content is lower than that found in nerves. The horns of the spinal cord, like nerves, contain proteins moving towards the cathode (Figure 23).

We also determined the electrophoretic mobility of soluble proteins from the nervus olfactorius and the nervus lienalis of cows and found that these nerves also contain a considerable amount of albumin. The nervus lienalis was freed from the connective tissue of the epineurium, which enabled us to detect the presence of albumin in the nerve fibers. These nerves did not contain proteins which moved towards the cathode.

Electrophoretic studies on proteins from the ganglion spinale have shown the presence of both globulins and albumins. The latter were present both in the nerve fibers and in the connective tissue.

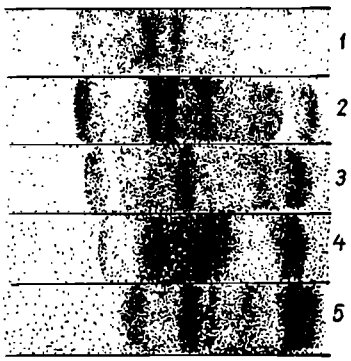

FIGURE 23. Electrophoretic diagrams of soluble proteins from the white matter of cow spinal cord (1), horns of the spinal cord (2), sciatic nerve of adult cow (3), sciatic nerve of cow embryo (4), and cow serum (5).

We have also determined the electrophoretic mobility of the following nerve proteins from the autonomic nervous system: the truncus sympathicus (which consists of nerve fibers and nerve ganglia), and the ganglion stellatum (which consists of nerve cells and nerve fibers). The electrophoretic diagrams showed that the truncus contained globulin and considerable amounts of albumin. Similar results were obtained for the ganglion stellatum. Neither the spinal cord ganglia, the truncus sympathicus, nor the ganglion stellatum contained proteins moving towards the cathode. The only differences in the protein composition of the above formations were in their globulin content.

We have isolated nerve albumin (by zone electrophoresis) and compared it with serum albumin. The electrophoretic mobilities of both albumins were identical. Nerve albumin is also similar to serum albumin in the concentration of ammonium sulfate necessary for its precipitation. Thus, the available data show that the two albumins resemble each other very closely.

Electrophoretic studies on brain and sciatic nerve proteins of cow embryos (2, 4, and 7 months old) have shown that the nerves differ from the brain in that they contain considerable amounts of albumin and of proteins moving towards the cathode. The protein composition of the brain and nerves becomes more complex as the embryo develops. Thus, protein differentiation appears to accompany the functional development of nerve tissue.

To summarize, the above electrophoretic studies on the soluble proteins of the central and peripheral nervous systems have shown the following: 1. The soluble proteins of the central nervous system consist of globulins, or of proteins with the electrophoretic mobility of serum globulin. 2. The peripheral nerves contain globulins, a considerable amount of albumins and proteins moving towards the cathode. 3. The spinal cord also contains albumins and proteins moving towards the cathode. 4. Nerves, as well as various sections of the autonomic nervous system contain albumin, but do not contain proteins moving towards the cathode. 5. Albumin was found in nerve fibers and was shown not to be contamination by connective tissue protein. 6. During embryonic life the proteins of the central nervous system and the peripheral nerves become differentiated as the nervous system develops.

The main limitation of paper electrophoresis is that the proteins become adsorbed on the filter paper (especially lipoproteins), which inhibits their migration.

We therefore decided to use agar-gel as a supportive medium for electrophoresis instead of paper.

Agar-gel contains 98.5% buffer solution and does not prevent the migration of proteins in the electric field.

We found that with agar-gel we could obtain a much better resolution (a larger number of fractions) of the soluble proteins of the nervous system than we were able to obtain with paper. We succeeded in resolving the soluble brain proteins into 16 fractions, and even these proved to be nonhomogeneous.

When soluble brain proteins were precipitated with ammonium sulfate and then subjected to agar-gel electrophoresis, an even large number of fractions were obtained. Precipitation with various concentrations of ammonium sulfate yielded fractions which differed in their protein composition.

In further investigations we used the combined method of ammonium sulfate fractionation and agar-gel electrophoresis /20/. The results obtained with this method showed that the proteins present in the gray and white matter of the cerebral hemispheres are identical. The only exception was the protein fraction precipitated with saturated ammonium sulfate (60–70%). In the gray matter this fraction was resolved on electrophoresis into seven components, while in the white matter it was resolved into six components.

Generally, only quantitative differences were found between proteins of the gray and white matter.

The separation of proteins by agar-gel electrophoresis into various fractions enabled us to study their enzymatic activity. We have succeeded in detecting enzymatic activity in various protein fractions. In other words, we studied the localization of various enzymes in different electrophoretic protein fractions.

We have also succeeded in purifying various enzymes by combining electrophoresis with other methods of protein purification, such as chromatography on Sephadex, or on cellulose derivations. In this way we were able to obtain enzymes which were electrophoretically homogenous (such as brain protease).

For a considerable period of time, before the advent of isotopes, brain proteins were considered as metabolically inactive compounds. Since the brain has a high metabolic activity, it was thought that this characteristic property was due to an active carbohydrate metabolism.

The first determinations of the incorporation of radioactive amino acids into brain proteins showed that the incorporation was slow. This strengthened the belief that brain proteins were inactive, even though Greenberg showed at that time that there was a rapid incorporation of radioactive methionine into brain proteins, provided that the amino acid was administered directly into the brain.

Further investigations showed that the low rate of incorporation of radioactive amino acids into brain proteins was due to the blood–brain barrier, which prevented the entry of amino acids into the brain. When radioactive amino acids were injected intracisternally they were shown to be incorporated at a rate which was comparable to that observed in the liver. These results were obtained by Gaitonde and Richter, who injected radioactive methionine subarachnoidally and by Waelsch and Lajtha, who injected radioctive lysine intraperitoneally. Lysine penetrates rapidly into the brain and has an advantage over methionine and glycine in that

it is not converted to other amino acids in short-term experiments. Similar results were also obtained by Vladimirov, et al., who confirmed that brain proteins exist in a dynamic state and undergo rapid turnover. Analogous results were obtained in experiments on the incorporation of radioactive phosphorus into phosphoproteins.

Experiments on the rate of incorporation of amino acids into brain proteins were usually carried out by determining the relative specific radioactivity of the proteins following the administration of the radioactive amino acid. The relative specific radioactivity of proteins is the ratio of the specific radioactivity of proteins in brain tissue to that of the trichloracetic acid extract. It is a reliable measure of the rate of amino acid incorporation into brain proteins.

The turnover rate of a protein may be evaluated from the rate of amino acid incorporation into the protein. The term "half-life" of a protein is used to designate its turnover rate. Lajtha and Waelsch found that the half-life of brain proteins increases with the time interval elapsing between the introduction of the radioactive amino acid and the sacrificing of the animal.

In their experiments mice were sacrificed from two minutes to one hour after radioactive lysine was administered to them. When the time interval between the administration of the radioactive amino acid and sacrifice was two minutes, the half-life was 2.8 days. If the time interval was 60 minutes, the half-life was 15.2 days.

The dependence of the half-life of proteins on the time interval which elapses between the administration of radioactive amino acids and the sacrificing of the animal is conditioned by the presence in the brain of various proteins with different rates of turnover. There are proteins with a very high turnover (microsomal proteins) as well as proteins with a long half-life, such as proteolipids, neurokeratin, and myelin sheath proteins. In experiments of short duration (in which the time that elapses between the administration of C^{14}-lysine and the determination of radioactivity is short) the half-life of proteins with a high turnover, or short half-life, is measured. In experiments of long duration the half-life of more stable proteins is determined. In young animals, whose brains undergo rapid growth and differentiation, the content of proteins with a long half-life is low.

The metabolic heterogeneity of brain proteins has been shown by Kravchinskii and Silich, of the Institute of Biochemistry of the Academy of Sciences of the Ukrainian SSR, and by Vladimirov, who determined the rate of incorporation of radioactive methionine and tyrosine into various fractions of brain proteins. The fractions were obtained by extraction with water, sodium chloride, and sodium hydroxide. Metabolically active protein fractions could be extracted with water and sodium chloride. Less active proteins were extracted with alkali or remained in the alkali-insoluble residue.

Inasmuch as the brain consists of numerous functionally and structurally different parts, the problem arises as to the turnover rate of proteins of various parts of the brain.

Studies of various investigators (Palladin and Vertaimer, Cohn, Gaitonde and Richter, Furst, Lajtha and Waelsch, Vladimirov, Pogodaev and Nefedova) have shown that the most metabolically active proteins

(those with the shortest half-life) are found in the cerebellum and the cerebral cortex. The proteins of the spinal cord have the longest half-life. However, these studies were made on the total protein content of various parts of the central nervous system, and it is known that each part contains different protein fractions with different turnover rates. Moreover, brain gray matter proteins have a considerably higher rate of turnover than those from the white matter.

Lajtha and Waelsch calculated the half-life values of proteins from various sections of the brain and spinal cord of monkeys (M a c a c a). They found that the spinal cord proteins had the longest half-life, followed by those of the medulla oblongata, thalamus, hypothalamus, cerebellum, and cerebral cortex. Proteins with the shortest half-life were found in the corpus callosum. These are proteins of the white matter in which the cellular elements consist mainly of glia.

These rather surprising results may be explained by the fact that the half-life values were calculated from measurements of the time-dependent changes in the specific activity of a radioactive amino acid (lysine) in the free amino acid pool and in the protein. In these calculations it was assumed that there is a homogeneous pool of free amino acids and that these soluble amino acids are the protein precursors. Any erroneous measurement in the specific radioactivity of the precursor leads to an erroneous half-life value. The free lysine in the white matter of the corpus callosum may not be in a homogeneous pool and may constitute only a small fraction of the protein precursor. A revision in the calculations should show that the proteins of the corpus callosum have a longer half-life than that found.

Numerous investigators (Greenberg, Gaitonde and Richter, Palladin, Belik and Krachko, Lajtha and Waelsch) have found that the turnover rate of brain proteins decreases with age. These results were also confirmed by Bulankin and Parina. In one-month-old rats the half-life of brain proteins was 1.2 days, while in older rats it was 3.6 days. The decrease in protein turnover may be due to the age-dependent accumulation of proteins with low activity. This hypothesis would concur with the findings of Lajtha that the brain of adult mice contains proteins with a long half-life which are absent in young (10-day-old) animals. It is also strengthened by the finding that brain proteins of young and adult animals differ in their amino acid composition.

The white and gray matter of embryo brains differ in their amino acid composition. (Mechislavov). During embryonic and early postembryonic development the content of amino acids in the gray and white matter of guinea pig brain changes.

Polyakova, studying brain and nerve proteins of variously aged embryos, showed that the complexity and differentiation of the soluble proteins increases with embryonic development.

In order to study the turnover of proteins from various intracellular components of brain tissue, Furst, Lajtha, and Waelsch determined the rate of incorporation of C^{14}-lysine into proteins of various subcellular fractions of the cerebral cortex of monkeys. The subcellular fractions were isolated by differential centrifugation. They found that here, as in other tissues, the highest rate of incorporation was displayed by the microsomal proteins.

Similar results were obtained by Palladin, Belik, and Krachko, who studied the incorporation of radioactive methionine into the proteins of nuclei, mitochondria, microsomes, and the cytoplasmic soluble fraction obtained from the cerebral hemispheres and cerebellum of cats. The highest protein turnover was found in the microsomes, and the lowest in the mitochondria. Protein turnover in the nuclei was intermediate. In general, protein turnover was higher in fractions from the cerebellum than in the corresponding cerebral fractions.

Clouet and Richter showed that the microsomal proteins have the highest rate of C^{14}-methionine incorporation. Upon fractionation of the microsomal proteins C^{14}-methionine was incorporated most rapidly into the fraction obtained by high speed centrifugation. This fraction contained microsomal lipids, and nucleic acids and ribosomes.

Clouet and Richter, determining protein radioactivity five hours after the injection of radioactive methionine, found that the radioactivity of the microsomal nucleoproteins gradually decreased with a concomitant increase in the specific radioactivity of proteins of other subcellular fractions. On the basis of these data Clouet and Richter assumed that microsomal proteins are transient and are rapidly transferred to proteins of other fractions.

Furst, Lajtha, and Waelsch studied the incorporation of radioactive lysine into the subcellular fractions obtained from functionally different regions of the brain and found that the microsomal proteins have the most rapid incorporation. Thus, the microsomes contain proteins with the shortest half-life. Their turnover is about one hour or less.

The highest protein content was found in the mitochondria (about 40% of the total tissue protein). A somewhat smaller amount was found in the soluble cytoplasmic fraction. Microsomes and nuclei had the lowest protein content.

Since each subcellular fraction apparently consists of more than one protein species, an attempt was made to separate these proteins into various fractions and to study their turnover. Belik fractionated the proteins from the soluble cytoplasmic fraction of the brain by ammonium sulfate precipitation and determined the turnover rates of the various protein fractions obtained. The protein fraction precipitated by 20% saturation with ammonium sulfate had the highest turnover rate. The fractions precipitated in the range of 50-100% ammonium sulfate saturation had the lowest turnover rate. Proteins were also separated by acetone fractionation. These proteins which remained in solution in 50% acetone had the highest rate of turnover.

Thus it was shown that different proteins from the same subcellular fraction have different turnover rates.

The highest rate of brain protein synthesis takes place in the microsomes. This has been shown both in experiments in vivo and in vitro. On the other hand, protein degradation takes place mainly in the mitochondria. The studies of Polyakova, Belik, and Tsaryuk have shown that the highest protease activity (cathepsin) is present in the mitochondrial fraction. Protease activity is much lower in the microsomes and nuclei, and lowest in the soluble fraction; 80% of cathepsin activity is found in the mitochondria, 10% in the soluble fraction, 4% in the nuclear fraction, and 3% in the microsomal fraction.

The rate of amino acid incorporation into proteins of various brain structures has also been measured by means of histoautoradiography. Fischer, Kolouśek, and Lodin, and Kolouśek and Lodin, in experiments on dogs, measured the incorporation of radioactivity into proteins of the motor neurons from the anterior horns of the spinal cord and into cells of the granular layer of the cerebellum. In experiments on rabbits and cats they found that the rate of metabolism differs in neuronal proteins from various regions of the central nervous system. The highest rate was found in Purkinje's cells of the cerebellum. It was lower in the motor neurons of the horns of the spinal cord, and lowest in the neurons of the cerebral cortex, Flannigan and McClean, working with rats, found that the rate of C^{14}-methionine incorporation varies in different brain sections. The rate of incorporation into proteins of the cerebellar cortex and subcortical formations was very high.

According to the data of Schultze, Oehlert, and Maurer, the rate of protein turnover in nerve cells is 53-70 times higher than that in glia cells. The highest turnover was found in the large pyramidal cells of the cortex and in the cells of Ammon's horns. In cells of the cortical sensory centers protein metabolism was less rapid than in cells of the motor and autonomic centers.

It appears that the data obtained by biochemical and histoautoradiographic methods are to some extent contradictory, The data obtained from biochemical studies on the high rate of protein turnover in phylogenetically young structures of the brain (cerebral cortex) were not corroborated by histoautoradiographic data, which show that protein turnover in nerve cells of the phylogenetically younger formations is lower than in neurons of older formations, such as the spinal cord. At the same time, the rate of protein turnover is very high in cells of the ganglion layer of the cerebellum, which is phylogenetically young formation. It should be recalled that autoradiograms show only the localization of radioactive amino acids in the cells, so that this method cannot be used for studies on turnover rates.

The peripheral nerves differ from brain tissue in their protein composition. It has been shown that they also differ in their rate of protein turnover. According to Silich, of the Institute of Biochemistry of the Academy of Sciences of the Ukrainian SSR, the rate of incorporation of radioactive methionine into nerve proteins is much lower than into brain proteins, in both the white and the gray matter.

Various fractions of nerve proteins, extracted with water, sodium chloride, or sodium hydroxide showed different rates of protein turnover. The rate of protein turnover in nerves was lower than that of the corresponding protein fractions in the brain.

Thus, the different functions of the peripheral nerves and of the central nervous system are correlated with differences in protein composition and turnover rate. It is logical to assume that in the central nervous system there is a higher prevalence of proteins with a high metabolic rate and short half-life, whereas in the peripheral nerves the reverse situation should be true.

More recent investigations indicate that substances can move along nerve axons. Hebb and Waites have shown that cholineacetylase disappears from the distal part of the nerve with a concomitant increase in enzymatic activity in the proximal part of the nerve, which is indicative of the movement of

the enzyme along the nerve axon from the cell where it is formed to the nerve ending where it functions. To ascertain whether this assumption is correct Lajtha studied protein metabolism in the peripheral nerves by determining protein radioactivity in various segments of the nerve following the administration of radioactive amino acids. The data obtained were inconclusive.

It would be of great interest to elucidate the correlation between the functional activity of the brain and its protein metabolism. Several attempts have been made in this direction. Soula studied proteolysis in the brain under various conditions of increased and decreased proteolytic activity. He determined the total nitrogen content of the brain, the content of amino acid nitrogen, and the ratio of amino acid nitrogen to total nitrogen for which the term "aminogenesis coefficient" was coined. This coefficient was used for calculations of the extent of proteolysis or aminogenesis.

At first Soula determined the extent of aminogenesis in brains of normal animals (dogs and rabbits) under normal conditions. Thereafter he studied the effect of various agents which enhanced or inhibited nervous activity on aminogenesis. Among the former were heat, faradization, asphyxia, curare, strychnine, and cocaine. Among the latter were low temperature, chloroform, chloralosis, chloral hydrate, morphine, and ether. It was shown that all enhancement of brain activity led to an increase in the rate of aminogenesis or, in other words, to increased proteolytic activity. During low brain activity aminogenesis decreased. These results permit one to conclude that the proteins of the nervous system constitute an integral part of the brain and that brain activity is accompanied by enhanced protein degradation.

In other investigations, changes in brain activity were induced by electrical stimuli or by various pharmacological drugs. It was feared that the action of such nonphysiological stimuli may cause not only physiological, but also pathological and morphological changes.

This prompted Gorodisskaya to design experiments in which changes in the activity of various brain sections were induced by phytiological agents. She studied the effect of physiological changes in the activity of the optical centers of the cerebral cortex on protein degradation (proteolysis). Changes in the activity of the optical centers were produced by sewing up the eyelids of young cats. These studies showed that the rate of proteolysis in the optical zone of the cerebral cortex of cats in resting states (cats with sewn-up eyelids) was always lower than in the optical centers of control animals. In blind cats no changes in the rate of proteolysis were found in other cortical centers, such as the motor or the sensory centers. The optical tracts of normal cats showed a higher rate of proteolysis than those of blind cats. A similar correlation between changes in activity and protein metabolism has been established for the auditory centers. An increase in the activity of the auditory centers was accompanied by an increase in protein hydrolysis.

These studies provided the first experimental evidence for the existence of a correlation between changes in the functional state of the cerebral cortex and changes in its metabolic activity.

Hyden, in experiments which provided the first evidence for metabolic activity of brain proteins, studied protein metabolism in cells of the anterior horns under conditions of exhaustion, and in cells of the spinal ganglion

after electrical stimulation. He concluded that during functional excitation protein metabolism increases. Anabolic processes prevailed under conditions of moderate excitation, whereas catabolic processes prevailed during strong excitation which led to exhaustion.

The use of radioisotopes proved fruitful for the elucidation of changes in brain metabolism during excitation. However, the results obtained were contradictory. Some investigators, such as Nechaeva, Palladin, Belik and Krachko, Zakharov and Orlyanskaya found that the rate of protein turnover in the central nervous system increased under conditions of excitation, such as stimulation of the skin receptors or the administration of phenamine. Other investigators (Vladimirov and Urinson, Gaitonde and Richter) have shown that during electrical stimulation there is a decrease in the rate of incorporation of radioactive amino acids into brain proteins.

Rozengardt and Maslova found that the rate of protein turnover decreased in the brain of rabbits which were subjected for 60 minutes to convulsions induced by corazol and an electric current. No changes in the rate of protein turnover were found during phosphacol-induced convulsions of two-hour duration in rats.

According to Pogodaev, et. al., the rate of brain protein metabolism changes during repeated epileptic fits, depending upon the number and frequency of the fits. The higher the intensity of the epileptic fits, the lower the rate of protein metabolism. Pogodaev assumed that excitation induced by phenamine or sound stimuli is accompanied by an increased rate of protein hydrolysis due to the enhanced activity of proteolytic enzymes, and by an increased rate of protein synthesis. However, the main observation made during excitation was that carboxyl groups were released due to deamination. Koloušek found a decreased rate of protein turnover during epileptic fits elicited with pharmacological drugs. Dingman, et al., found no changes in protein turnover during convulsions.

According to Vladimirov the rate of phosphoprotein turnover increases during excitation which results from nonconditioned and conditioned reflexes. According to Zakharov and Orlyanskaya the turnover of phosphoproteins increases during convulsions elicited with cardiamine. Rozengardt and Maslova did not find any change in phosphoprotein turnover during convulsions elicited with corazol (in rabbits) and phosphacol (in rats).

Heald studied the effect of electrical stimulation on the rate of incorporation of radioactive phosphorus into brain slices. The results were ambiguous at first. He concluded that electrical stimulation increases the turnover rate only of the phosphoproteins present in the nuclear fraction but has no effect on phosphoproteins in the soluble fraction and in the mitochondria.

The contradictory results obtained by several investigators who studied brain protein metabolism during excitation with the aid of radioactive isotopes may be due to a number of reasons. In such experiments it is of great importance to estimate correctly the state of the nervous system. It is also important that experimental excitation should be closely related to physiological excitation. Excitation elicited with very strong stimulants may result in convulsions. If the stimulation is prolonged excitation may pass to overexcitation and to inhibition, which is indicative of the exhaustion of the nervous system. In experiments with radioisotopes knowledge of the extent of the endogenous pool needed for the determination of the relative

specific activity is of great importance. The rate of incorporation of radioactive amino acids should be measured at various time intervals following their administration. The presence of a blood-brain barrier should also be accounted for. Finally, all experiments should here appropriate controls.

The majority of investigators have found that during excitation of the nervous system which resembles physiological excitation the rate of protein turnover increases. During very strong excitation which leads to convulsions, or during prolonged excitation, which may lead to over-excitation and exhaustion of the nervous system, protein turnover decreases.

Rozengardt and Maslova compared the nature of biochemical changes with objectively recorded states of the central nervous system and of other systems of the organism. They established that the general state of the organism (and particularly its respiration and circulation) plays an important role in these changes. They found that in all experiments during which the blood pressure did not fall the metabolic rate remained unchanged during excitation. In experiments in which the blood pressure fell and remained at a low level the rate of protein turnover decreased. They consider blood pressure as a factor which reflects the state of brain blood circulation, and hence the transport of glucose and other substances to the brain. A deficiency in these substances results in a decrease in the synthesis rate of energy-rich compounds in the brain, with a concomitant decrease in the rate of protein synthesis.

On the basis of these data Rozengardt and Maslova proposed that the factor which determines the rate of protein turnover in the central nervous system is not the prevailing state of excitation or inhibition but the supply of oxygen, glucose, and other metabolites to the brain.

In research on the inhibition of brain protein metabolism most investigators studied the effect of various narcotics on brain protein turnover with the aid of radioactive techniques. Here also, the results were contradictory. Gaitonde and Richter, Vladimirova and Urinson, Pogodaev and Nefedova, and Nechaeva, studying the incorporation of radioactive methionine and glycine into brain proteins during narcosis elicited with pentabarbiturate, ether, and amytal, found a decrease in the rate of protein metabolism. Other investigators (Fridman-Pogosova, Palladin, Belik and Krachko) using the anesthetics urethan and veronal, or a medinal-urethan mixture, found no changes in protein metabolism.

According to Pogodaev, both protein synthesis and degradation decrease but synthesis prevails over degradation. According to Shnyak, protein turnover increased (by 30%) in the cerebral cortex of rats during exhaustive excitation, and decreased (by 48%) during 30 minutes of deep sleep of which followed exhaustive excitation.

According to Rozengardt and Maslova, narcotic sleep has no effect on the metabolism of phosphoproteins.

During excitation, apart from changes in protein metabolism, numerous authors detected changes in brain protein configuration and structure, in physicochemical properties of proteins, and in the rate of protein decomposition. Promyslov found that during prolonged narcotic sleep there is a decrease in protein content in rabbit brain.

According to Unger et al., the number of protein sulfhydryl groups increases during excitation, which indicates a structural rearrangement of the proteins. This is in accordance with the postulated role of sulfhydryl groups in nervous activity.

According to Martinson et al., excitation is accompanied by the deamidation of proteins and by an increase in their electrophoretic mobility. During prolonged and strong electrical stimuli (the electrodes were placed on the head) amidation supercedes deamidation, which lowers protein of electrophoretic mobility; the state of excitation passes into that of inhibition.

Similar phenomena were observed in proteins of the sciatic nerve. Excitation was accompanied by deamidation (change in protein macrostructure) and increased electrophoretic mobility. During inhibition amidation prevailed and the electrophoretic mobility decreased. Analogous changes were observed during sleep under sedation. According to Gershenovich changes in amide groups (both labile and nonlabile) can be observed during oxygen poisoning, during which the functional state changes from sleep to strong convulsions, depending on the extent of poisoning. During severe hypoglycemia, caused by the administration of insulin, Tyakhepyl'd observed protein deamidation, which led to alterations in protein structure.

The rate of protein turnover in the subcellular fractions of brain tissue in young rabbits decreases considerably during starvation. The most prominent decrease is in the mitochondrial proteins and, to a lesser extent, in the microsomal proteins and in proteins of the soluble cytoplasmic fraction. The smallest effect was found in nuclear proteins (Smerchinskaya). Thus, starvation affects protein synthesis in the structural elements of brain tissue.

During starvation the rate of proteolysis in the white matter of the brain increases markedly. The rate of proteolysis in the gray matter remains unchanged (Palladin and Gulyi). Protein deficiency is accompanied by an increase in proteolysis in rat brain nerve cells (Kuporenku).

Brain nitrogen metabolism is influenced also by the yearly seasons. In autumn the brain of adult rabbits contains more creatine than in the spring /23/. Similar differences in creatine content were observed in the brain of rabbit embryos; they contained more creatine in the autumn than in spring. Pigeon brain also contained different amounts of creatine in spring and in autumn. The rate of proteolysis in pigeon brain was higher in the spring than in the summer (Palladin and Gulyi).

It may be concluded that there is a definite connection between the functional activity of the nervous system and protein turnover, and that proteins have a definite function in the nervous system.

Brain proteins may be utilized as a source of energy under various conditions. Aboad has shown that excitation of the sciatic nerve leads to the utilization of proteins instead of carbohydrates.

Mullins found that excited nerves preferentially utilize glycine, alanine, and glutamic acid. Although a close connection has been established between the functional activity of the nervous system and protein metabolism, not much is known about the specific role of various proteins in the function of the nervous system. It is feasible that nerve tissue contains a number of proteins, each being specific for a different functional region of the nervous system.

An attempt to find such proteins was made by Turpaev, who succeeded in isolating a specific protein connected with a determined function of the nervous system. This is a choline receptor. He found that this protein is present in the water-soluble fraction of brain homogenates and that it can be precipitated by ammonium sulfate.

One of chief concerns in the biochemistry of the nervous system is the characterization of specific proteins; finding suitable methods of isolation, defining their metabolic activity, identifying them with a specific functional state of the brain, and finding their specific role in the function of the nervous system. Waelsch assumed that brain proteins are the main units of the storage of information, which is one of the specific functions of the nervous system.

The highest rate of protein turnover in the nervous system was found in regions rich in nerve cells. The highest rate of protein turnover is characteristic of the microsomal and ribosomal proteins. However, more stable proteins with a longer half-life also play an important role in the nervous system. Such are the enzymes, which are responsible for brain metabolism.

Studies on proteins of the central and peripheral nervous systems are necessary for the understanding of nervous system abnormalities (acquired and inborn), and particularly of psychic disorders. The latter depend on disturbances in protein metabolism, a redistribution of specific proteins, an increase or decrease in protein content, or the replacement of some amino acids by others. Changes in brain protein metabolism may provide the basis for its various pathological states.

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*BRAIN METABOLISM DURING HIBERNATION**

In hibernating animals (gophers) there is a physiological inhibition of all metabolic processes during hibernation and an increased activity of all metabolic processes upon awakening. These processes are of special interest in studies on brain metabolism; there are found situations of marked decreases or increases in the activity of the central nervous system which cannot be reproduced experimentally.

From the biological point of view hibernation is a unique adaptation of living organisms to low environmental temperature. Hibernation is a state of prolonged, rarely interrupted, deep rest in animals which during their active period of life are homothermic. Hibernation is characterized by extremely low rates of physiologic functions such as respiration, circulation, and excretion, by a marked decrease in the metabolic rate, and by a deep inhibition in the activity of the central nervous system.

Hibernating animals can be easily wakened by lowering or raising the environmental temperature. Artificial awakening is accompanied by a marked increase in the activity of all physiologic functions, including the activity of the central nervous system.

Studies on the brain metabolism of hibernating animals present possibilities for investigating the dynamic aspects of nervous activity metabolism.

In hibernating animals one can observe and study both strong inhibition and strong excitation of the nervous system. In view of this we, at the Institute of Biochemistry of the Academy of Sciences of the Ukrainian SSR, have attempted to study the brain metabolism of hibernating animals (gophers) during various periods of their lives.

All the data /8/ have shown that during hibernation the total content of nitrogenous substances, as well as the content of nonprotein nitrogen remains unchanged. During prolonged and deep sleep an increase is observed in the total content of brain phosphorus, while the amount of acid-soluble phosphorus decreases somewhat. At the same time, the content of lipid phosphorus increases slightly.

Studies have shown /8/ that the content of residual nitrogen and the ratio of residual to total nitrogen during hibernation decrease to a greater extent in the gray matter of the cerebral hemispheres than in the white matter or in the cerebellum. The content of orthophosphoric acid remains unchanged both in the gray and in the white matter of the cerebral hemispheres, but decreases in the cerebellum.

The total content of phosphates in the gray and white matter of the brain decreases. The same is true for the phosphate content of the cerebellum,

* Lecture read at the Fourth International Biochemical Symposium held in St. Wolfgang, Austria, in 1952. Appeared in "Comparative Biochemistry," edited by D. Richter, 1964, pp. 131-138.

but to a lesser extent. The content of acid-insoluble phosphorus decreases to a greater extent in the gray matter than in the white. Thus, during hibernation the content of all brain phosphorus compounds decreases. This is due to the decrease in the content of acid-insoluble phosphorus fraction (phosphatides).

In artificially awakened gophers /7/ the ammonia content of the brain increases. It can also be shown /4/ that during hibernation the rate of brain tissue respiration decreases. Expenditure of glucose also decreases.

We have also investigated /5/ the metabolism of RNA, phosphoproteins, and phospholipids in the brain of hibernating animals. Experiments were conducted on awake, hibernating, and artificially awakened gophers (four hours before the experiment). For comparison, experiments were also conducted on gophers during pharmacological sleep of 24 hours' duration. In all these experiments the brain and spinal cord were assayed for the rate of incorporation of radioactive phosphorus, which was administered to the animals four hours prior to sacrifice (0.1 mc/kg of body weight).

These studies have shown that considerable differences exist in the rate of incorporation of radioactive phosphorus into the RNA, phosphoproteins, and phospholipids of the brain and spinal cord in awake, hibernating, and artificially awakened animals. The specific activity (the number of radioactive disintegrations per mg of phosphorus) of the various phosphorus containing compounds in the brain and spinal cord of hibernating gophers was by several orders of magnitude lower than that found in awake animals. Thus, during hibernation the turnover of RNA, phosphoproteins, and phospholipids decreases, as compared with control animals.

The rate of incorporation of radioactive phosphorus into various components of gopher brain during sodium-medinal-induced narcotic sleep of 24 hours' duration was similar to that observed during hibernation, but the extent of the decrease in the rate of incorporation was much less pronounced.

Of special interest were the data obtained with artificially awakened animals. During awakening the rate of radioactive phosphorus incorporation into nucleic acid, phosphoproteins, and phospholipids increases but did not attain the rate present in awake animals.

Thus, during the inhibition of central nervous system activity, observed during hibernation, there is a decrease in the turnover rate of ribonucleic acid, phosphoproteins, and phospholipids. The turnover rate of these substances increases in awake and artificially awakened animals.

Experiments were also conducted on the rate of incorporation of radioactive carbon into the brain glycogen of gophers. These studies have shown that glycogen metabolism is also lowered during hibernation.

We have studied the rate of protein turnover in hibernating animals, which manifest a marked decrease in body temperature and a deep inhibition of central nervous system activity, in alert animals, and in artificially awakened animals in which a comparatively rapid rise in body temperature and an increase in physiological function is noted. Investigations were conducted on the rate of radioactive methionine incorporation into brain proteins of gophers /2/. Hibernating and artificially awakened animals were studied in January and March, while awake animals were studied in June.

Radioactive methionine was introduced subcutaneously 18 hours prior to sacrifice (10,000 counts per gram of body weight). Methionine was

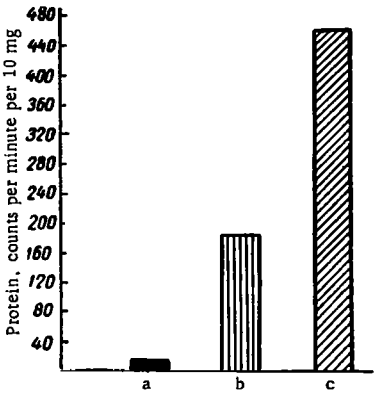

FIGURE 24. Radioactivity of total proteins in gopher brain

a — hibernation; b — awake animals; c — artificially awakened

administered to hibernating, awake, and artificially awakened gophers (to the latter immediately after awakening, caused by raising the environmental temperature).

The rate of radioactive methionine incorporation into total brain proteins was determined (by measurement of protein specific activity) during hibernation, wakefulness, and after awakening. These studies have shown that during hibernation, which can be considered a process of prolonged and deep inhibition of brain activity, there is a low rate of methionine incorporation into brain proteins; in other words, the rate of protein turnover is low. In several experiments with animals in a state of deep sleep the turnover rate was practically zero (Figure 24).

Artificial awakening of the gophers was accompanied by a sharp increase in nervous activity; the rate of protein turnover in the brain increased 25-fold over that of sleeping animals.

In awake gophers the rate of protein turnover was lower than in those artificially awakened. The specific radioactivity of brain proteins was by several times higher than in hibernating animals, but considerably lower than in those artificially awakened.

In order to determine whether the differences in the rate of methionine incorporation are due to differences in the permeability of the blood brain barrier during various functional states, we measured the specific radioactivity of the acid soluble brain tissue fraction during hibernation, wakefulness and artificial awakening. This fraction can be considered as a unique metabolic pool from which brain cells obtain the building blocks for metabolic synthesis.

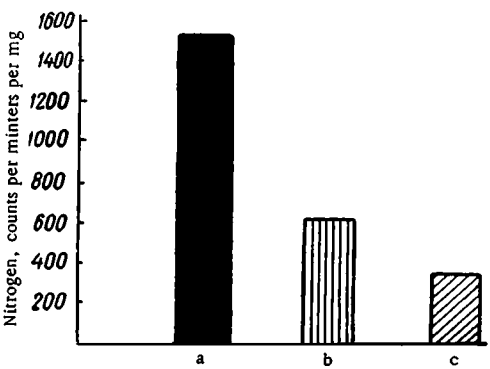

FIGURE 25. Radioactivity of the acid soluble fraction in gopher brain tissue

a — hibernation; b — awake gophers; c — artificially awakened

These determinations showed that the highest radioactivity is found in the acid-soluble fraction of the brain of hibernating gophers.

The metabolic pool of awake and artificially awakened animals was considerably lower (Figure 25).

Thus, it may be assumed that the above-mentioned differences in the rate of protein metabolism are due to the different rates in protein metabolism and not to changes in the permeability of the blood brain barrier.

It is known that during artificial hypothermia there is a considerable decrease in the rate of protein metabolism in various tissues, including brain. In our Institute we have shown /3/ that hypothermia in rabbits results in a decrease in the rate of methionine incorporation into brain proteins, especially into proteins of the gray matter of the cerebral hemispheres.

In view of the recent interest in the biochemistry of subcellular constituents, we studied the effect of hibernation on protein synthesis in various subcellular fractions of brain tissue. We determined the rate of incorporation of radioactive methionine into the proteins of nuclei, heavy and light mitochondria, microsomes, and the soluble cytoplasmic fraction in hibernating, awake, and artificially awakened gophers. The subcellular fractions were obtained by differential centrifugation. In these experiments awake animals were studied during September—October.

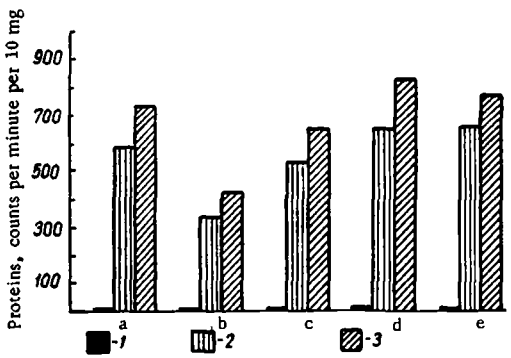

FIGURE 26. Radioactivity of proteins from subcellular fractions of gopher brain

a — nuclei; b — heavy mitochondria; c — light mitochondria; d — microsomes; e — soluble cytoplasmic fraction. 1 — hibernation; 2 — awake animal; 3 — artificially awakened animals.

The lower specific activity of all subcellular fractions studied was found in hibernating animals. It was twice as high in artificially awakened animals, and higher by several orders in awake animals. Within each group of animals (hibernating, awake, and artificially awakened) the rate of methionine incorporation into proteins of the various subcellular fractions differed. The highest rate of incorporation was found in microsomal proteins and in the soluble cytoplasmic fractions. It was somewhat lower in the nuclear proteins and still lower in mitochondrial proteins, especially those of the heavy mitochondria (Figure 26).

Determinations of radioactivity in the acid-soluble fraction showed that the highest radioactivity is found in hibernating gophers.

Thus there are different rates of brain protein metabolism in hibernating, awake, and artificially awakened animals. These differences are found not only in total brain proteins but also in various subcellular fractions. The lowest rate of protein turnover, both of total protein and of various subcellular fractions, is found in hibernating animals. It is considerably higher in awake animals, and even higher in those artificially awakened. These studies have also shown that the rate of protein turnover differs in the various subcellular fractions. In all the functional states studied (hibernation, wakefulness, and artificial awakening) the highest rate of protein turnover was found in the microsomes; the rate of nuclear protein turnover was lower, while the lowest rate was found in mitochondrial proteins. Of great interest is the high rate of protein metabolism in the

brain and its subcellular fractions in artificially awakened gophers as compared with awake animals.

Difficulties were encountered in determining the rate of protein turnover during inhibition, and more so during excitation of the central nervous system in various animals. Various investigators obtained contradictory results in studying protein metabolism during excitation and inhibition of the central nervous system induced by various pharmacological drugs and other nonphysiologic agents, such as electrical stimulation.

Studies on hibernating animals enabled us to elucidate the effect of the main physiological states of the central nervous system — excitation and inhibition — on the metabolism of various brain substances, and particularly of proteins, phosphoproteins, nucleic acids, phospholipids, and glycogen.

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*LOCALIZATION OF SOME ENZYMES IN SUBCELLULAR FRACTIONS OF THE BRAIN AND IN VARIOUS PROTEIN FRACTIONS OBTAINED BY ELECTROPHORESIS**

Recent biochemical and physiological investigations have been concerned with the subcellular components of various tissues. Studies on the functional biochemistry of cells play an increasing role in the development of various biological sciences.

Since all metabolic processes are directed and controlled by enzymes, enzymatic studies from various approaches are of main importance in the field of biochemistry. The coordinated activity of various enzyme systems cannot be understood without a knowledge of the subcellular distribution and localization of the enzymes.

In view of this, our main concern has been the study of the distribution of enzymes in the different subcellular components of brain tissue. In the present communication I would like to present the results of our studies on the distribution of several enzymes involved in nitrogen, carbohydrate, and phosphorus metabolism in subcellular brain components. These studies were conducted at the Institute of Biochemistry of the Academy of Sciences of the Ukrainian SSR. The distribution of the following enzymes was studied: proteinase, glutaminase, adenosine deaminase, adenylic acid deaminase, guanase, pyrophosphatase, adenosinetriphosphatase, phosphoglucomutase, and aldolase. We have investigated the localization of these enzymes in nuclei, mitochondria, microsomes, and the soluble cytoplasmic fraction of brain tissue.

Adult rabbit brains were used for the experiments. The various subcellular fractions were isolated by differential centrifugation according to the modified method of Brodie and Bain.

Enzymatic activity in a given fraction was calculated per mg of protein and per total protein content. The protein content of all subcellular fractions was determined. These determinations showed again that the highest protein content is found in the mitochondrial fraction.

Since brain protease has not been studied extensively, we first decided to investigate protease activity in various sections of the central nervous system of cattle and rabbits /5/. The highest proteolytic activity was found in the gray matter of the cerebral hemispheres and the cerebellum — the two functionally most complex sections. It was lower in the white matter, and lowest in the spinal cord.

We next studied protease distribution in various subcellular fractions of nerve tissue. These studies showed /5/ that the highest protease activity is found in the mitochondrial fraction. It is considerably lower in

* Revised lecture read at the Hungarian Academy of Sciences, 1961. "Acta Physiologica Academiae Scientiarum Hungaricae", Vol. XXI, 1962, p. 106-111, and at the Fifth International Biochemical Congress, August 1961. Moscow.

microsomes. In mitochondria protease activity is three times higher than in the microsomes. In the nuclear fraction it is somewhat lower than in the microsomes. The lowest protease activity is found in the soluble cytoplasmic fraction, being one-seventh to one-eighth that of the mitochondrial fraction (Figure 27).

To obtain an idea on the total content of enzymes in the various subcellular fractions, we determined the total protein content of each fraction studied. It was found that the mitochondrial and soluble fractions have the highest protein content. The protein content of nuclei is one-third as high and that of the microsomal fraction, one-seventh.

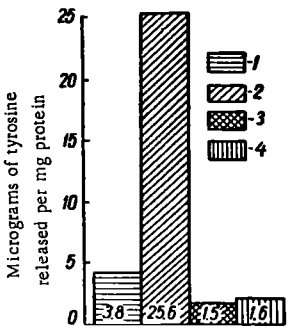

FIGURE 27. Protease activity in subcellular fractions of the brain

1 — nuclei; 2 — mitochondria; 3 — microsomes; 4 — soluble fraction

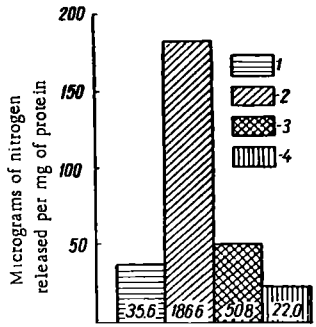

FIGURE 28. Glutaminase activity in various subcellular fractions of the brain

1 — nuclei; 2 — mitochondria; 3 — microsomes; 4 — soluble fraction

Based upon the activity of protease in the various subcellular fractions and upon the total protein content, we calculated the enzyme content of each fraction in percent of total protease activity. The result showed that the mitochondria contain 80% of total protease activity. The remaining protease activity is distributed among the soluble fraction (about 10%), nuclei (4%), and microsomes (about 3%).

Glutaminase acitivity was studied in cattle and rabbit brains /4/. It was found that the glutaminase of the central nervous system is localized mainly in the gray matter of the cerebral hemispheres (about 60% of total glutaminase activity). About 35% of total glutaminase activity was found in the cerebellum, and only about 8% was found in the white matter of the cerebral hemispheres.

Studies on glutaminase activity in various subcellular fractions have shown that the highest glutaminase activity is found in the mitochondria, followed by the microsomes. The nuclear and soluble fractions have the lowest glutaminase activity (see Figure 28).

The glutaminase content was calculated in each of the fractions as percent of total glutaminase activity. Glutaminase was found to be localized mainly in the mitochondria (about 90%). The soluble fraction contained 8%, and nuclei, 3.5% of the total glutaminase activity. Glutaminase activity in the microsomes was negligible.

Thus, glutaminase and protease are localized mainly in the mitochondrial fraction.

Studies on the distribution of adenosine deaminase gave different results. /7/. It was shown that the white matter of the cerebral hemispheres had the highest adenosine deaminase activity. Gray matter activity was one third as high, and that of the cerebellum and spinal cord even lower. The lowest adenosine deaminase activity was found in the sciatic nerve.

Studies on adenosine deaminase activity in various subcellular fractions showed that the soluble fraction had the highest enzymatic activity. Nuclei, mitochondria, and microsomes all displayed similar enzymatic activity, which was about one-twentieth that of the soluble cytoplasmic fraction (Figure 29). Thus, about 90% of the total adenosine deaminase activity is localized in the soluble fraction. The distribution of guanine deaminase is similar to that of adenosine deaminase.

Studies on adenylic acid deaminase have shown /7/ that the deamination of adenylic acid in the brain is catalyzed by two enzymes: one catalyzes the dephosphorylation of adenylic acid to adenosine (5'-nucleotidase), the other catalyzes the deamination of adenosine (adenosine deaminase). In all subcellular fractions 5'-nucleotidase is present. Microsomes show the highest nucleotidase activity; it is also quite high in nuclei and mitochondria. Nucleotidase activity is low in the cytoplasmic fraction (Figure 30).

The two enzymes, 5'-nucleotidase and adenosine deaminase catalyze the deamination of adenylic acid in the white matter of the cerebral hemispheres, in the cerebellum, and in the spinal cord. The gray matter of cerebral hemispheres contains, in addition, adenylic acid deaminase, which catalyzes the direct deamination of adenylic acid without preliminary dephosphorylation.

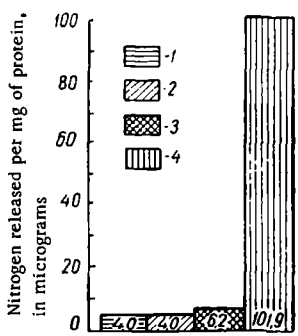

FIGURE 29. Adenosine deaminase activity in subcellular fractions of the brain

1 — nuclei; 2 — mitrochondria; 3 — microsomes; 4 — soluble fraction

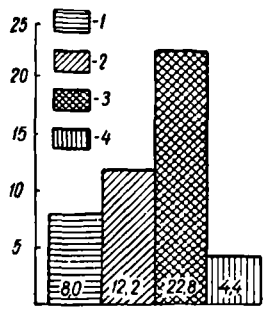

FIGURE 30. 5'- nucleotidase activity in various subcellular fractions of the brain (in micrograms of phosphorus per mg protein)

1 — nuclei; 2 — mitochondria; 3 — microsomes; 4 — soluble fraction

Guanase activity is higher in the brain than in other tissues. Studies on guanase activity in functionally different brain sections /9/ have shown that the gray matter of the cerebral hemispheres has the highest guanase activity. Guanase activity is lower in the white matter of the cerebral hemispheres and lowest in the cerebellum (one-half as high as in the gray matter of the cerebral hemispheres). Thus the distribution of guanase in the various brain sections differs from that of adenosine deaminase.

Studies on the distribution of guanase in the subcellular brain fractions have shown that the soluble fraction has the highest guanase activity. Guanase activity in mitochondria is one-tenth that of the soluble fraction, and even lower in the microsomes and nuclei (see Figure 31).

Calculations showed that about 90% of guanase activity is localized in the soluble cytoplasmic fraction; 7% of the total activity is found in the mitochondria, about 5% in the microsomes, and 0.1% in the nuclei.

The highest pyrophosphatase activity was found in the gray matter of the cerebral hemispheres and in the cerebellum. The lowest activity was found in the sciatic nerve. Intermediate values were obtained in the midbrain, medulla oblongata, spinal cord, and the white matter of the cerebral hemispheres /11/.

In order to study the distribution of pyrophosphatase in various subcellular fractions we isolated the nuclei, mitochondria, microsomes, and the soluble cytoplasmic fraction from the gray and white matter of the cerebral hemispheres, cerebellum, midbrain, medulla oblongata, spinal cord, and sciatic nerve and determined pyrophosphatase activity in these fractions.

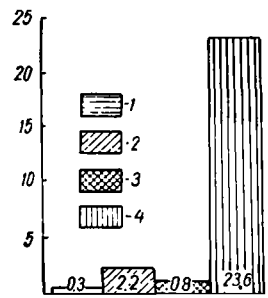

FIGURE 31. Guanase activity in various subcellular fractions of the brain (in micrograms of ammonia nitrogen per mg protein)

1 — nuclei; 2 — mitochondria; 3 — microsomes; 4 — soluble fraction

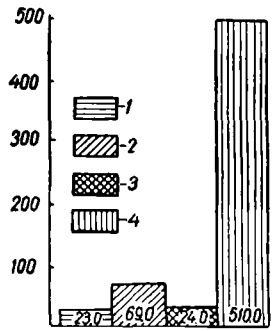

FIGURE 32. Pyrophosphatase activity in various subcellular fractions of the brain (in micrograms of phosphorus per mg protein)

1 — nuclei; 2 — mitochondria; 3 — microsomes; 4 — soluble fraction

Comparative studies on pyrophosphatase activity in various subcellular fractions from various sections of the nervous system have shown that

the cytoplasmic fractions obtained from the brain hemispheres, cerebellum, and midbrain, have the highest pyrophosphatase activity. The lowest activity was found in the sciatic nerve. Intermediate activity values were found in the cytoplasmic fraction from the medulla oblongata and spinal cord.

Calculations showed that the soluble cytoplasmic fraction from various sections of the nervous system contains about 90% of the total pyrophosphatase activity. Pyrophosphatase activity is very low in particulate fractions: in the mitochondria it is about 4-8%; in microsomes, 0.5-1.5%; and in the nuclei, less than 0.5%.

Our first studies /2/ on adenosinetriphosphatase activity in the subcellular fractions of nerve tissue showed that the highest activity was found in the microsomes (Figure 33). These data contradicted those obtained by Aboad and Chekh. The reason for the contradiction was that we prepared our homogenates in sucrose solution containing calcium chloride ions, which activate adenosinetriphosphatase, especially in the microsomal fraction.

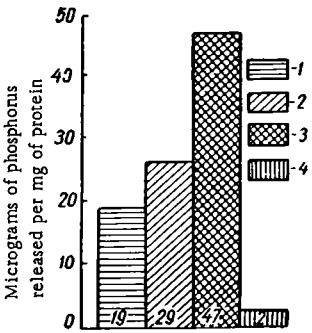

FIGURE 33. Adenosinetriphosphatase activity in various subcellular fractions of the brain (in sucrose solution containing calcium ions)

1 — nuclei; 2 — mitochondria; 3 — microsomes; 4 — soluble fraction

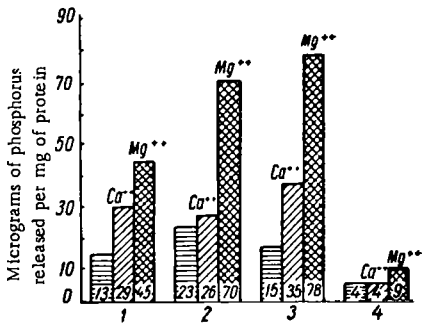

FIGURE 34. Adenosinetriphosphatase activity in subcellular fractions of the brain in the presence and absence of calcium and magnesium ions

1 — nuclei; 2 — mitochondria; 3 — microsomes; 4 — soluble fraction

In subsequent experiments we used sucrose solutions without calcium. Under these conditions the highest adenosinetriphosphatase activity was found in the mitochondria. It was considerably lower in the nuclei and microsomes, and even lower in the cytoplasmic fraction (Figure 34). Studies on the effect of calcium and magnesium ions on adenosinetriphosphatase activity have shown that various subcellular fractions contain adenosinetriphosphatase with different properties (Figure 34).

The highest phosphoglucomutase activity was found in the gray matter of the cerebral hemispheres, and the lowest was found in the white matter (the cerebellum had intermediate values) /10/. Phosphoglucomutase was also found in the soluble cytoplasmic fraction. The soluble cytoplasmic fraction had the highest specific acitivity of phosphoglucomutase. Activity

was low in the particulate fractions (Figure 35). Thus, about 90% of the total phosphoglucomutase activity was found in the soluble cytoplasmic fraction, and only 10% in the remaining subcellular fractions.

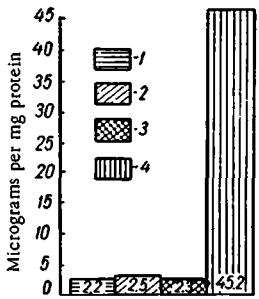

FIGURE 35. Phosphoglucomutase activity in subcellular brain fractions

1 — nuclei; 2 — mitochondria; 3 — microsomes; 4 — soluble fraction

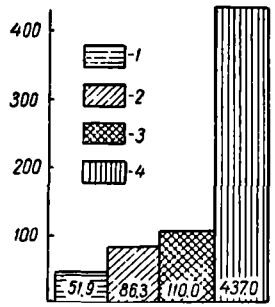

FIGURE 36. Aldolase activity in subcellular brain fractions

(in micrograms of phosphorus per mg protein):

1 — nuclei; 2 — mitochondria; 3 — microsomes; 4 — soluble fraction

The highest aldolase activity was found in the gray matter of the cerebral hemispheres and in the cerebellum. Aldolase activity was much lower in white matter of the cerebral hemispheres and in the medulla oblongata. The distribution of aldolase among the various subcellular brain fractions is similar to that of phosphoglucomutase. Among the subcellular fractions, the highest specific aldolase activity was found in the soluble fraction. Aldolase activity was considerably lower in cellular components such as nuclei, microsomes, and mitochondria (Figure 36). About 80% of the total aldolase activity was found in the soluble cytoplasmic fraction, 13% in the mitochondria, about 3% in the microsomes, and about 2% in the nuclei.

These studies have shown that different distributions of enzymes exist in different subcellular brain components. Protease, glutaminase, 5'-nucleotidase, and adenosinetriphosphatase are localized in the particulate fractions, mainly in the mitochondria (the highest activity of 5'-nucleotidase was found in the microsomes). Adenosine deaminase, guanase, pyrophosphatase, as well as the glycolytic enzymes, phosphoglucomutase and aldolase, are localized mainly in the soluble fraction of the cytoplasm.

Having succeeded in separating the soluble brain proteins, by means of agar-gel electrophoresis, into 12-16 fractions /6/, we attempted to study the localization of some of the brain enzymes in these fractions.

Since only soluble proteins may be studied by electrophoresis, this method was useful only for the study of proteins from the soluble cytoplasmic fraction, or of loosely bound proteins which could be easily extracted and solubilized. Because of this, the following enzymes could be studied by this method: adenosine deaminase, guanine deaminase, aldolase, and phosphoglucomutase, all of which are localized in the soluble cytoplasmic fraction. We also study the distribution of protease, which is readily extractable from mitochondria with distilled water.

Cattle brains were used in these experiments. Agar-gel electrophoresis was carried out on two glass plates which were immersed in agar solution. Troughs on both plates contained solutions of lyophilized brain protein. After electrophoresis the proteins in the narrow plate were fixed with acetic acid, dried, and stained with "amido black 10B." In this way we were able to obtain chromatograms of soluble brain proteins.

The agar-gel in the broad plate was cut into 1-cm wide strips; 17 strips were obtained in all. These strips were transferred into test tubes, frozen in dry ice, and thawed. The liquid which separated during this procedure was filtered off. The filtrate was used for determinations of protein concentration and enzymatic activity.

Table 10 shows the specific activity of protease, adenosine deaminase, guanase (guanine deaminase), aldolase, and phosphoglucomutase in all 17 strips. It can be seen from Table 10 that the enzymes studied are present in different strips. Protease and phosphoglucomutase are located in the strips to the left from the site of application; that is, closer to the cathode. Adenosine deaminase and aldolase are found in strips to the right of the site of application; that is, closer to the anode. Guanase is located almost at the site of application, in the 7th and 6th strips.

Figure 37 shows a chromatogram presenting the localization of various enzymes found in different strips, as tabulated in Table 10.

TABLE 10. Distribution of enzymes during electrophoresis on agar gel

No. of strip	Enzymatic activity				
	protease, in mg of tyrosine per mg protein	adenosine deaminase, in mg of ammonia nitrogen per mg protein	guanase, in mg of ammonia nitrogen per mg protein	aldolase, in mg of phosphotriose per mg protein	phosphoglucomutase, in mg of phosphorus per mg protein
1	0	0	0	0	0
2	0	0	0	0	0
3	0	0	0	100.0	0
4	0	66.6	0	761.1	0
5	0	113.5	8.2	2030.4	0
6	24.1	16.7	23.1	453.7	0
7*	121.6	0	26.9	0	0
8	337.0	0	0	0	0
9	828.0	0	0	0	0
10	1426.0	0	0	0	161.2
11	1583.0	0	0	0	908.8
12	206.0	0	0	0	472.7
13	0	0	0	0	0
14	0	0	0	0	0
15	0	0	0	0	0
16	0	0	0	0	0
17	0	0	0	0	0

* Site of application.

It can be seen from the figure that aldolase and adenosine deaminase are located mainly in the fourth protein fraction. Guanase is present in proteins which were not well separated during electrophoresis and were therefore found at the site of application. Protease is found in the 8th, 9th and 10th fractions. Phosphoglucomutase is located mainly in the 10th protein fraction. It has also been detected in the 9th fraction.

Thus, aldolase and adenosine deaminase show the highest electrophoretic mobility. Phosphoglucomutase shows the lowest electrophoretic mobility; protease moves close to phosphoglucomutase. Guanase has an intermediate electrophoretic mobility. It can be seen that various enzymes have different electrophoretic mobilities, which enabled us to separate them by agar-gel electrophoresis.

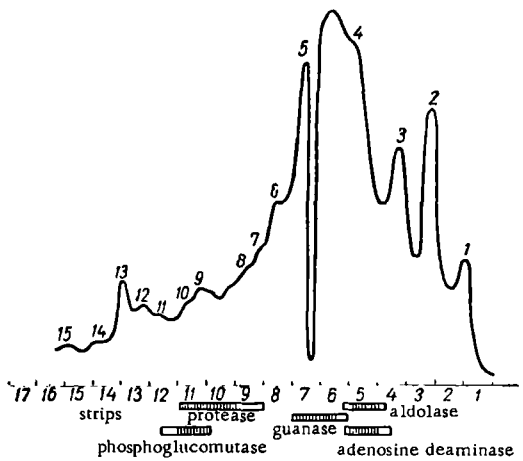

FIGURE 37. Localization of enzymes in protein fractions obtained by electrophoresis

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*BRAIN BIOCHEMISTRY AND PSYCHOCHEMISTRY**

One of the most important problems in the biochemistry of the nervous system is the elucidation of the metabolic processes underlying the functional activity of the higher sections of the central nervous system which are involved in psychic activity.

There is no doubt that psychic activity is closely associated with the higher brain sections, and particularly with the cerebral cortex. The relationship between the metabolic processes of the cortex and its functional activity have not been closely investigated and remains, for the most part, obscure. Without a precise knowledge of cortical metabolism we cannot understand the problems of psychochemistry, whose ultimate goal is to elucidate the relationship between the manifestations of psychic life and the various biochemical processes taking place in the brain.

I had already spoken on this topic in 1922 /1/ and discussed the data available at that time on the dynamic and functional biochemistry of the brain. At that time I had already mentioned that there were indications of a certain correlation between the chemical processes of the cortex and its functional activity, and particularly its psychic activity. These data were the first in the field of psychochemistry.

The available data on the chemical composition of various brain sections indicate that the cerebral cortex is richer in proteins than any other section, including the gray matter of the subcortical ganglia. This indicates that proteins have an important function in the psychic activity of the cerebral cortex.

Our studies and those of other Soviet and foreign biochemists have shown that the rate of protein turnover in the cerebral cortex is one of the highest /2, 3/.

The cerebral cortex is very rich in ribonucleic acids, which have a very high rate of turnover /4/. Also characteristic of the cortex is a very high glycogen content and an extremely active carbohydrate metabolism /5, 6/.

In the cerebral cortex of dogs, the turnover rate of phospholipids is higher than in other brain sections. In rabbits, the cerebral cortex is at a considerably lower level of functional development and, therefore, its rate of phospholipid metabolism is not higher than in other brain sections /4/.

Another approach to the investigation of brain chemical processes is the study of enzyme activity. Our studies /7/ have shown that the cortex possesses the highest activity of enzymes involved in carbohydrate and nitrogen metabolism (phosphorylase, hexokinase, aldolase, glutaminase, and adenosinetriphosphatase), as well as in oxidation-reduction reactions (cholinesterase, cytochrome oxidase, and succinic acid dehydrogenase /8/).

* Lecture read at the First Congress of the Ukrainian Biochemical Society, held on June 11, 1965, in Chernovtsy.

Thus, the cortex is characterized by a high content of proteins and nucleic acids, a high rate of protein, nucleic acid, and carbohydrate metabolism, and high enzymatic activity.

Many scientists who studied the effect of various factors in brain activity have concluded that proteins play an important role in cortical function.

Soula /9/, as early as 1913, studied proteolysis, or as he called it aminogenesis, in the brain under various conditions of increased nervous center activity (heat, faradization, strychnine, and cocaine), or decreased activity (low temperature, chloroform, chloral hydrate, morphine, and ether). He found that increased activity of the cortical centers is accompanied by aminogenesis, or enhanced proteolysis. A decrease in cortical activity was always accompanied by decreased aminogenesis. These data led him to conclude that the activity of the cortical centers is associated with protein metabolism.

Gorodisskaya /10/ studied proteolysis in the optical centers of the cerebral cortex of cats. She found that upon the transition of the optical centers from relative rest to enhanced activity there is an increase in protein metabolism.

One of the main problems in the area of the biochemistry of the nervous system is to determine the biochemical processes underlying the various psychic states in man, to be able to control them in cases of psychic disorders. According to Pavlov, this will become possible only when we understand the chemical processes underlying cerebral function.

To this end comprehensive biochemical studies of the brain are needed. The metabolism of all compounds present in the brain should be investigated, in order to understand brain function and the relation between metabolic processes and higher nervous activity. Since the main functional states of the nervous system are excitation and inhibition, the central problem in the biochemistry of the nervous system is brain metabolism during excitation and inhibition of higher nervous activity.

We have studied certain aspects of brain metabolism in animals with experimentally produced inhibition or excitation of higher nervous activity. Inhibition was elicited with various pharmacological drugs. Depending upon the nature of the drug and the duration of its administration we were able to obtain various degrees of inhibition, from sleep to deep narcosis.

Our data and those obtained by numerous other scientists have shown that during inhibition of nervous activity (during narcotic sleep) the turnover rate of ribonucleic acid, phosphoproteins, phospholipids, and glycogen decreases. At the same time the content of ATP and glycogen increases. During deeper inhibition the rate of protein turnover decreases.

Investigations have shown that during sleep synthesis prevails over decomposition, facilitating the restoration of brain activity (protective inhibition).

Excitation was elicited with pervitin, widely used in medical practice as a stimulant of higher nervous activity, and with cardiazol. It had been shown that these stimulants differ in their effect on brain metabolism due to their different physiological activity. Excitation elicited with pervitin results in rapid synthesis of ATP in the brain. This, apparently, is because pervitin stimulates the central nervous system, increasing the period of alertness and eliminating tiredness. Cardiazol stimulates the cerebral cortex but, unlike pervitin, does not increase the working capacity of the brain.

These were the first data which explained the different physiological effects of different stimulating drugs.

Our experiments, as well as those of other scientists /22/ who studied brain metabolism during excitation of the nervous system, have shown that during excitation the content of brain glycogen decreases, the rate of glycolysis increases, and the turnover rate of nucleic acids, proteins, phosphoproteins, and phospholipids also increases.

The above studies touched upon the effect of pharmacological drugs on brain metabolism and revealed the biochemical basis for the physiological effects of the different drugs. These studies belong to the field of neurochemistry, whose ultimate concern is the control of biochemical processes during psychic disorders.

This is the concern not only of neurochemistry but also of psychopharmacology, a branch of biochemical pharmacology, whose ultimate aim is to control the biochemical processes underlying the various psychic states in man by means of pharmacological drugs.

Neurochemists and psychopharmacologists have recently become interested in certain monoamines present in nerve tissue, such as serotonin and the catecholamines (adrenaline, noradrenaline, and dopamine).

Catecholamines play a unique role in neurohumoral regulation and in nervous system function. They are frequently referred to as hormones-mediators. Adrenaline is a hormone, whereas noradrenaline is a mediator. Dopamine is a precursor of noradrenaline and adrenaline and, according to various scientists, plays an important role in the activity of the central nervous system. It is now believed that serotonin and the catecholamines are closely associated with the functional states of the nervous system, and that disturbances in monoamine metabolism may play an essential role in the development of nervous and psychic disorders.

Many severe and frequently encountered disorders of the nervous system or psyche result from genetic defects in the biosynthesis of certain enzymes. In particular, it is known that psychic disturbances may be caused by a defect in the biosynthesis of an enzyme involved in alanine metabolism in the brain.

Psychiatrists consider that changes in catecholamine metabolism play an important role in the pathochemical characterization of disturbances in motor reactions and muscle tonus during Parkinsonism and in disturbances in the emotional sphere during schizophrenia.

Numerous psychopharmacological drugs used against psychoses selectively inhibit those enzymes involved in the metabolism of physiologically active amines (monoamines and acetylcholine).

The monoamines of the nervous system are decomposed by monoamineoxidase. Psychopharmacological or neurotrophic drugs which inhibit monoamineoxidase also inhibit the decomposition of monoamines in the brain. These substances are among the most important modern psychopharmachological drugs.

Monoamineoxidase inhibitors are neutropic drugs of the antidepressant type; they are used for the treatment of depressive states. They activate higher nervous activity, increase motor activity, and improve the general well-being. They are antagonists of neuroplegic preparations, such as reserpine.

The use of monoamineoxidase inhibitors against various psychic disorders is one example of the utilization of biochemical knowledge by biochemical parmacology. Reactions catalyzed by monoamineoxidase can be affected by the use of pharmacological drugs.

The chief representative of monoamineoxidase inhibitors is iprazid, or iproniazid, which has been used in psychiatry since 1953 for the treatment of depressive states.

Iprazid prevents the decomposition of serotonin and noradrenaline, thus causing the accumulation of these amines in the brain. The content of serotonin increases much more rapidly than that of noradrenaline.

Studies have been conducted on the effect of iprazid on monoamineoxidase and on serotonin and catecholamine metabolism /12/. Little is known about the effect of iprazid on other aspects of brain metabolism. This problem should be studied systematically since the effect of iprazid cannot be explained solely by its inhibitory action on monoamineoxidase.

We have conducted several investigations in this direction; we have studied the effect of iprazid on nitrogen and carbohydrate metabolism on the content of monoamines, and on the activity of monoamineoxidase in the brain. These studies should also be helpful in understanding the role of serotonin and the catecholamines in the organism. We have also studied the effect of serotonin on certain aspects of brain metabolism.

These investigations were conducted by the scientific staff of the Laboratory of the Biochemistry of the Nervous System at the Institute of Biochemistry of the Academy of Sciences of the Ukrainian SSR, which includes the following people: E.E. Goncharova, Ya.T. Terletskaya, V.I. Kocherga, M.D. Kurskii, E.P. Gotovtseva, L.S. Smerchinskaya, A.A. Musyalkovskaya, A.N. Fedorov, O.N. Zryakov, and the head of the Laboratory of Biochemical Pharmacology, Professor S.E. Baluev.

In order to elucidate the effect of iprazid on brain nitrogen metabolism we investigated its effect (in rabbit brain) on the nitrogen content of ammonia; on the content and metabolism of the amide groups of glutamine and of proteins; and on the activity of glutaminase, glutamine synthetase, and monoamineoxidase.

These studies have shown /13/ that the administration of iprazid to rabbits (subcutaneous injection) results in an increase in the serotonin content and a decrease in the glutamine content. The decrease in glutamine content is apparently not due to changes in the rate of synthesis or utilization of glutamine, since there were no changes in the activity of glutamine synthetase or of glutaminase.

It has been shown that the administration of glutamic acid to rabbits given iprazid can restore brain glutamine content to normal. Hence, it has been advised that for the treatment of depressive states iprazid should be administered together with glutamic acid, so as to avoid a decrease in glutamine content.

Iprazid has no effect on the nitrogen content of ammonia and on the amide nitrogen content of proteins.

To study the turnover of brain nitrogen fractions we administered N^{15}-ammonium chloride to rabbits and measured its rate of incorporation into these fractions. It was found that iprazid has no effect on the content of ammonia or of amide groups in proteins, but that the turnover rate of these substances increases.

It should be mentioned that the decreased rate of N^{15} incorporation into the amide groups of glutamine was observed under conditions when the concentration of N^{15} in the metabolic pool was low, which apparently accounts for our results. It must be concluded that iprazid has no effect on glutamine metabolism.

Iprazid apparently affects the turnover of ammonia in the central nervous system. Iprazid stimulates the incorporation of ammonia into proteins, which may be deduced from the high turnover rate of labile amide protein groups in the brain and from the lowered concentration of N^{15} in ammonia.

The majority of workers explained the effect of iprazid on brain metabolism and on the functional state of the brain by the inhibition of monoamineoxidase, which results in the accumulation of serotonin. To ascertain whether the accumulation of serotonin affects brain nitrogen metabolism, we studied the effect of serotonin on brain metabolism. Rabbits were injected with serotonin intracranially so as to bypass the hematoencephalic barrier. The results showed that serotonin has no effect on brain nitrogen metabolism.

It may be assumed that the absence of change in nitrogen metabolism upon the administration of serotonin is due to the destruction of serotonin by monoamineoxidase. To test this we administered serotonin by monoamineoxidase. To test this we administered serotonin to animals in which monoamineoxidase activity was inhibited by iprazid. Here, too, no changes in nitrogen metabolism were observed.

According to the available data the inhibition of monoamineoxidase in rabbits is accompanied by an accumulation of serotonin and noradrenaline. In cats and dogs only the serotonin content increases, while that of noradrenaline remains unchanged.

In this connection, we investigated the effect of serotonin on brain nitrogen metabolism in cats, and found no observable changes. Thus, if the effect of iprazid depends upon changes in monoamine content due to the inhibition of monoamineoxidase, it may be concluded that it is the concentration of noradrenaline which increases, and not of serotonin.

As has been mentioned before, iprazid brings about different accumulations of the different monoamines. Serotonin content increases to a larger extent than that of the catecholamines. More important is the fact that serotonin accumulates at a faster rate, as may be seen from a comparison of the rates of synthesis: the rate of serotonin synthesis is much higher than that of noradrenaline.

It is thought /14/ that the excitation observed in animals upon repeated administration of iprazid is due to the accumulation of noradrenaline and not of serotonin. In our experiments on rabbits excitation developed gradually, and only when the noradrenaline concentration increased markedly.

All these studies indicate that the effect of iprazid on nitrogen metabolism cannot be explained solely on the basis of brain serotonin accumulation. It is possible that the effect of iprazid on nitrogen metabolism is due either to iprazid itself or to its metabolic products.

We have studied the effect of serotonin on the turnover of brain proteins /15/. It was found that the intracranial administration of serotonin to rabbits leads to a decrease in the rate of methionine incorporation into all brain proteins.

An increase in the content of endogenous serotonin, due to the administration of iprazid, leads to a decrease in the rate of protein turnover (of total brain proteins as well as of proteins in the various subcellular fractions). Upon the simultaneous administration of iprazid and serotonin such a decrease is even more pronounced. It may therefore be assumed that the effect of iprazid on the turnover rate of brain proteins is the result of a high serotonin content in the brain. It is true that iprazid also affects the content of noradrenaline, but to a lesser extent.

Since the serotonin content differs in various sections of the central nervous system, we thought that it might be of interest to elucidate the effect of iprazid on the rate of protein turnover in different brain sections /16/. The rate of protein turnover was determined by measuring the rate of incorporation of radioactive lysine into brain proteins. It was found that under the influence of iprazid, which was administered 17 hours prior to the determinations, the rate of lysine incorporation decreased. The greatest decrease in protein turnover was found in the pons varolii and the tubera quadrigemina. It has also been shown that under the influence of iprazid there is a selective increase in the permeability of the hematoencephalic barrier towards radioactive lysine.

Since the brain contains no glycogen reserves and its chief source of energy is glucose, we thought that it would be interesting to see whether the accumulation of monoamines would affect brain carbohydrate metabolism.

Our studies showed /17/ that a single administration of iprazid produced a 2—5-fold increase in glycogen content in the brain of rabbits. The glucose content also increased slightly. A more marked effect on glucose content was found upon repeated administrations of iprazid (for 4 days); the content of glycogen also increased. The activity of hexokinase decreased slightly.

Since in rabbits the administration of iprazid leads to an increase in the content of serotonin and noradrenaline, whereas in cats only the content of serotonin increases, we decided to study the effect of iprazid on glycogen and glucose content in cat brains. These studies showed that there was an insignificant increase in glycogen content and a small increase in glucose content. Hence, the increase in glycogen content in rabbits cannot be due only to the accumulation of serotonin. It has also been shown that when serotonin was administered to rabbits intracranially, no increase in glycogen content was observed.

We have also studied the effect of iprazid on the content of free nucleotides in the brain /18/. It was found that in rabbits iprazid had almost no effect on the content of ATP and ADP. In cats the content of ATP, ADP, and GTP increased; in dogs it decreased slightly. The intracranial administration of serotonin to rabbits brought about an increase in free brain nucleotides.

In contrast to rabbits, iprazid had no effect on glycogen content in the brain of dogs and cats. Hexokinase activity and glucose content also remained unchanged.

The data obtained by us were insufficient to explain satisfactorily the mechanism of iprazid action on the biochemical processes of the brain and on its functional states. Neither could they explain the effect of iprazid on monoamine metabolism, nor the significance of its inhibition of monoamineoxidase. We therefore studied the effect of another antidepressant, transamine, on these same biochemical processes.

Transamine is also an inhibitor of monoamineoxidase but it not a derivative of hydrazine /19/.

Both iprazid and transamine inhibit monoamineoxidase. According to our data, iprazid inhibits monoamineoxidase activity in mitochondria by 80%, while transamine inhibits activity by 70%. Both monoaminooxidase inhibitors bring about an increase in serotonin content in dog brain; iprazid by 70%; transamine by 90%. Noradrenaline content increases only upon the administration of transamine.

Comparative studies on the effect of iprazid and transamine on brain metabolism (in dogs and rabbits) have shown that the effects of the two drugs differ, sometimes in opposed directions. Iprazid brings about an increase in brain glycogen content, while transamine brings about a decrease. Glutamine content decreases after the administration of iprazid, but remains unchanged upon the administration of transamine.

Iprazid brings about a decrease in the content of glutamine amide nitrogen, whereas the nitrogen of the labile amide protein groups remains unchanged. Transamine has no effect on the content of labile amide groups of brain proteins, nor on the amide groups of glutamine. In rabbits both iprazid and transamine bring about a decrease in glutamine content.

Iprazid brings about 30% increase in ammonia content in dog brain. Tansamine has no effect on ammonia content.

Transamine brings about a decrease in brain glycogen content, whereas iprazid has the opposite effect. It may therefore be concluded that the accumulation of glycogen after the administration of iprazid is not the result of monoamineoxidase inhibition but is due to the direct effect of iprazid, or its metabolic products, on carbohydrate metabolism.

Both of the inhibitors of monoamineoxidase, iprazid and transamine, have different effects on the functional state of the central nervous system. The administration of iprazid to dogs brings about a state of motor unrest, aggressiveness, excitation of the subcortical formations of the brain, and enhancement of bioelectric activity. Transamine causes fear and unrest. It activates the brain biopotentials and leads to an increased rate of respiration.

Thus iprazid and transamine, both inhibitors of monoamineoxidase, have different effects on nitrogen and carbohydrate metabolism of the brain and on its functional state. It may be concluded that they have different modes of action and that their effect on brain metabolism cannot be due only to the inhibition of monoamineoxidase and, indirectly, to the accumulation of serotonin and catecholamines.

These investigations are still a long way from revealing the mechanism of action of iprazid and transamine, or the role of serotonin and noradrenaline in the brain. Further neurochemical and psychopharmacological studies are necessary in order to find the common biochemical denominator underlying the antidepressant activity of these neurotropic drugs.

The development of psychochemistry and psychopharmacology raised hopes that the pathogenesis of psychiatric disorders may soon be understood; and this particularly after the discovery of a large group of chemical compounds which produce various symptoms of psychic disorders. These are known as psychomimetic substances. With the discovery of psychomimetic substances it was hoped to obtain experimental conditions

which imitate human psychoses. To these compounds belong lysergic acid diethylamide, psilocibin, butatenin, and others.

Careful studies have shown, however, that these drugs are capable of producing only isolated symptoms or elementary syndromes which only remotely resemble various psychic disorders. None of the known psychomimetic compounds is capable of producing psychic disturbances resembling the common psychoses, such as schizophrenia or manic-depressive psychosis. It is therefore clear that the role of these compounds in studies on the pathogenesis of psychoses was grossly overestimated.

Nevertheless, these compounds have not been fully utilized in neurochemical and psychopharmacological studies. Further studies on the effect of these drugs on the biochemistry of the central nervous system and the functional states of the brain undoubtedly may be of great assistance in the fields of neurochemistry, psychopharmacology and psychochemistry. The investigation of psychomimetic drugs should therefore be continued.

One of the most important functions of the higher brain sections is the storage and recall of previously acquired information. We call this process memory. Man would be unable to exist without memory. All human behavior is determined by memory and experience. The problem of what is memory and what is the mechanism of its expression remains unsolved.

The word "memory" unites three separate concepts: the first is memorization — this is a process whereby a system passes from one state (section of the brain) into another; the second is the stabilization of the new state of the system, in other words, the imprinting of the new information; and the third is what we presently call recollection — this is the utilization of the imprinted information.

The storage and recall of information may arise from a number of changes: physical changes in the nerve cells, changes in the complex network of nerve fibers and synapses which connect the cells, and chemical changes in subcellular components.

At the beginning of the 20th century the prevailing theory was that memory is due to mechanical changes in the form and size of nerve cells. The discovery by Berger, in 1920, that the brain possesses electrical activity led to the electrical theory of memory. This theory was discredited by Leslie, in 1940, who showed that the mechanism of memory is based on fine chemical changes in various components of the nerve cell. Such changes must satisfy two conditions: the cell components must be extremely stable, and their structure should be such as to allow a large number of transformations.

Only three cell components satisfy these requirements: DNA, RNA, and proteins. DNA carries the genetic code. RNA molecules are formed on DNA templates and then serve as templates for protein synthesis. Since the memory acquired during a lifetime is not passed on to future generations, memory cannot be formed as a result of intracellular changes in DNA. This means that nerve cells store memory through changes in the RNA-protein complexes.

This hypothesis was confirmed by the studies of Dingman and Sporn, and others. Hyden showed that nerve cells contain extremely large amounts of RNA — ten times more than glia cells. The RNA of nerve cells differs from that of glia-cell RNA. Synthesis and decomposition of RNA in nerve cells proceed at a high rate. On the basis of these results Hyden assumed that RNA is somehow involved in the storage of information (memory).

From the biochemical point of view there is nothing impossible in Hyden's hypothesis. The hypothesis requires the existence of RNA molecules, acting as templates for protein synthesis, which will react to certain signals appearing in the cell.

The fact that RNA plays an important role in the activity of the higher sections of the nervous system has already been stressed. According to the data obtained by us and other investigators the cerebral cortex, the most highly organized part of the central nervous system, has the highest RNA content. There is no doubt that proteins also play an important specific role in higher nervous activity.

The high RNA content of nerve cells led Hyden to propose the hypothesis that the mechanism of memory is based on changes in the structure and nucleotide composition of RNA, with the subsequent formation of specific proteins.

Hyden /20/ studied the nucleotide composition of RNA from the nuclei and cytoplasm of Deiter's cells in trained rats. The rats were trained to maintain equilibrium on a thin metallic rod. He found that no changes occurred in the composition of the cytoplasmic RNA, as compared with control animals, but that the nuclear RNA of trained rats was richer in adenine and poorer in uracil. The A/U ratio changed from 1.06 to 1.35. At the same time the total amount of RNA increased. The training of rats was accompanied by a synthesis of specific RNA with a specific nucleotide sequence. Thus, the neurons of trained rats differed biochemically from those of control rats.

Hyden /21/ also studied the content and nucleotide composition of RNA in Deiter's and adjacent glia cells of trained rats. He found that both in glia cells and in neurons the total content of adenine increased, while that of cytosine decreased. The ratio of adenine to uracil (A/U) increased from 1.32 to 1.52.

It is of interest that nerve cells of the reticular formation, which do not participate in the training process, showed no changes in the nucleotide composition of RNA.

Hyden's experiments show that the changes occurring in the RNA of cells involved in the training process are highly specific. The fact that glia cells react in the same way as neurons is not surprising. It is known that these two types of cells comprise a single functional unit.

Hyden assumed that glial RNA is responsible for short-lived memory, since the branched membranes of glia are well adjusted to rapid processes. The RNA of neurons may be responsible for the storage of information. The investigations of other scientists also confirmed the importance of RNA in the storage of information.

The experiments which have recently been conducted in the USSR by Tongur confirm the hypothesis that RNA is the molecule which codes for memory. He has found that when ribonuclease is injected into the brain of trained mice, the mice forget their training. These results may be explained in the following way: the new RNA which is synthesized in the brain of mice during training is destroyed by ribonuclease. This leads to the loss of memory.

It is true that this problem is yet far from being solved. We do not know which RNA code is responsible for memory, how are nervous impulses transformed into biochemical reactions which lead to the synthesis of

specific RNA, what is the mechanism of memory, and what is the role of proteins. To solve these problems further systematic studies are needed.

However, biochemistry (and especially molecular biochemistry) is progressing very rapidly and we hope that many of these problems will be solved in the near future. What is needed is hard work and the belief in success.

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